SECTION 6

The patient with another primary diagnosis

CHAPTER 149

The patient with diabetes mellitus

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Historical background and perspectives

The first description of protein in the urine is found in the sixteenth century when, in 1527, Paracelsus gave a lecture in Basel 'On the milk of the kidneys', an observation followed by a similar finding, in 1664, described in a textbook on therapeutic approach to medicine published by Frederick Dekkers (Cameron, 2003). It was only in the eighteenth century that Domenico Cotugno of Bari, physician to the King of the Two Sicilies, described for the first time the presence of protein in the urine of diabetic patients (Schena, 1994); notably in 1836, with Richard Bright, proteinuria is put into context with renal disease.

In 1969, Harry Keen, at the time physician at Guy's Hospital in London, was the first to make the important observation, that later opened an intense area of research, on the presence of increased urinary albumin excretion rate (UAER) in some patients with either type 1 or type 2 diabetes (Keen et al., 1969).

In parallel to albuminuria in 1936, discussion on renal lesions observed in patients with diabetes led to the description, by Kimmelstiel and Wilson, of the characteristic nodular fibrotic lesions observed in the diabetic glomeruli (Kimmelstiel and Wilson, 1936b). Kimmelstiel and Wilson introduced the term diabetic nephropathy (DN) to define a clinical syndrome of arterial hypertension, overt proteinuria (or macroalbuminuria), and worsening kidney function (Kimmelstiel and Wilson, 1936a). After the pioneering studies of Andres and Fiaschi on renal function (Fiaschi et al., 1952) and histopathology (Fiaschi et al., 1959) of diabetic subjects, DN was later described as part of a more general diabetes presentation of microangiopathy by Lundbaek (1970). Diabetic glomerulopathy was initially considered as the histopathological counterpart of DN, but soon it became apparent that, in particular in patients with type 2 diabetes, this clinical syndrome could be associated also with changes of the nephro angiosclerotic type, or other primary glomerular diseases, or a mixture of different patterns (Gambara et al., 1993).

DN is currently the most feared diabetic chronic complication and is characterized by a progressive decline in glomerular filtration rate (GFR), eventually resulting in end-stage renal disease (ESRD). The all-cause mortality in patients with DN is nearly 20–40 times higher than that in patients without nephropathy. In recent years, it has become apparent that renal and cardiovascular diseases are closely related, and DN is acknowledged as an independent and powerful risk factor for cardiovascular disease (Karalliedde and Viberti, 2004). Most diabetic patients with chronic kidney disease (CKD) will die from cardiovascular disease before they reach ESRD and dialysis treatment (Berl and Henrich, 2006). Diabetes represents 30–40% of all patients receiving renal replacement therapy (Remuzzi et al., 2002b). The rising incidence and prevalence of CKD (GFR < 60 mL/min per 1.73 m^2) is today, like in the past, a major public health concern (Weening, 2004), and diabetes still represents the main determinant, also representing the major cause of ESRD (United States Renal Data System, 2010). The diabetes-related ESRD annual incidence rate has been decreasing since 1996 suggesting that treatments of risk factors (mostly hypertension) have significantly contributed to this reduction; nevertheless the pandemic of type 2 diabetes (Jones et al., 2005; Burrows et al., 2010) should be considered as a likely determinant for the predicted future increase in diabetes-related ESRD.

The prevention and management of diabetes and its renal complication remains a huge global challenge: the global number of diabetic patients is believed to be around 180 million and is set to increase to 350 million in the next two decades (Shaw et al., 2010). Type 2 diabetic patients will account for 90% of all cases, and we expect a dramatic increase in new cases of DN within the predicted 25–40% prevalence of renal disease in the diabetic population (Remuzzi et al., 2002b). The concomitant increase in associated cardiovascular mortality and morbidity, and ESRD, will have a significant social and economic impact, particularly in the developing world; in particular, some populations such as patients with Afro-Caribbean and Asian origin known to have a significant higher risk for renal disease.

Despite these considerations, recent studies clearly indicate that, at least within type 1 diabetes, 45% of patients still develop ESRD or die before the onset of ESRD (Forsblom et al., 2011; Rosolowsky et al., 2011), suggesting that there is still an urgent need to improve and refine our therapeutic targets and to use available and novel treatments more effectively.

Pathology

In the early phase of diabetic glomerulopathy (in studies in type 2 diabetic patients), in mildly/moderately injured glomeruli, an increase in vascular endothelial growth factor-A (VEGF-A)-mediated glomerular endothelial cell proliferation leads to augmented capillary length and diameter and expansion of glomerular volume (Hohenstein et al., 2006); this process is often paralleled by microaneurysm formation secondary to mesangiolysis (Paueksakon et al., 2002). At later stages of the disease, progressive

accumulation of extracellular matrix and glomerulosclerosis will result in a progressive loss of glomerular capillaries, despite new vessel formation (secondary to a hypoxic environment within the glomeruli) (Osterby and Nyberg, 1987).

Parallel early changes affecting the glomerular filtration barrier are characterized by podocyte foot process effacement and loss of podocyte in the urine (Bjorn et al., 1995; Pagtalunan et al., 1997; Meyer et al., 1999; Steffes et al., 2001; White et al., 2002; White and Bilous, 2004), and by reduction in glomerular endothelial cell fenestration (Toyoda et al., 2007).

At the level of the tubular interstitium, the major lesion is characterized by progressive interstitial fibrosis triggered by an early diffuse inflammatory cell infiltrate.

Macroscopic changes

The main macroscopic change observed in the diabetic kidney is an increase in kidney weight largely explained by tubular hypertrophy and interstitial expansion (Wolf and Ziyadeh, 1999; Vallon, 2011). These changes appear to be secondary to hyperfiltration (see 'Pathophysiology of microvascular damage') and active hyper-reabsorption of glucose, secondary to upregulation of the Na^+/glucose transporter sGLT2 (Hummel et al., 2011), that in turns leads to release of pro-inflammatory chemokines and alterations in proximal tubular salt and fluid reabsorption. The progressive tubular hypertrophy triggers a further increase in reabsorption of the glomerular filtrate and secondary worsening of hyperfiltration through the physiologic action of the tubule–glomerular feedback system (Vallon, 2011).

Microscopic changes (light and electron microscopy)

The glomerulus

The hallmark of diabetic glomerulopathy is diffuse mesangial area expansion, thickening of the glomerular basement membrane (GBM), and podocyte foot process effacement and fusion with detachment from the GBM.

Mesangial sclerosis appears to initially affect the glomerular tuft, progressing, at later stages of the disease, towards the glomerular capillaries resulting in progressive capillary obliteration and global glomerulosclerosis; importantly, mesangial expansion has been proposed as the central mechanism for loss of kidney function (Steffes et al., 1989). Pathognomonic presentation of the glomerular sclerotic process is accumulation of extracellular matrix into nodules firstly described by Kimmelstiel and Wilson (Kimmelstiel and Wilson, 1936b). These are often located at the periphery of the tuft and comprise acellular, eosinophilic, and lamellated structures; it is believed that they derive from obliterated capillaries and microaneurysms, or from focal mesangiolysis, which is followed by progressive matrix deposition which results in capillary obliteration and progressive diffuse complete glomerulosclerosis. The basement membrane may be in areas maintained in its structure like the normal one, or somewhat thickened, but is never wrinkled or split (Kimmelstiel and Wilson, 1936b).

Renal diabetic lesions in patients with type 1 diabetes are predominantly located in the glomerulus, but often include arteriolar, tubular, and interstitial lesions. In type 2 diabetes, the degree of glomerular lesions overlaps significantly with that of type 1 diabetes, but its presentation is more heterogeneous (Figs 149.1 and 149.2) (Dalla Vestra et al., 2000; White and Bilous, 2000). Previous studies have questioned whether the heterogeneous nature of renal lesions in type 2 diabetes could represent a different prognostic marker for progression towards ESRD (Gambara et al., 1993), but the magnitude of urinary protein excretion (clinical proteinuria), more than the pattern of glomerular lesions, appears to predict the risk of diabetic disease progression (Ruggenenti et al., 1998). Importantly, alterations in the glomerular vessels appear to be linked with the degree of albuminuria (Osterby and Nyberg, 1987; Osterby, 1992; Osterby et al., 1999).

The prevalence of non-diabetic renal disease among patients with type 2 diabetes mellitus varies widely depending on the selection criteria and the populations being studied, and ranges from 7% to 57% (Waldherr et al., 1992; Olsen and Mogensen, 1996; Soni et al., 2006; Huang et al., 2007; Ghani et al., 2009; Harada et al., 2012).

Thus, the usefulness of renal biopsy in diabetic patients is still heavily debated, particularly in the presence of hypertension and/or retinopathy. On the other hand, conducting a renal biopsy may be of paramount importance when the clinical scenario is not typical of DN. For instance, diseases other than diabetic glomerulopathy and/or nephroangiosclerosis are relatively frequent in elderly patients, such as most of those with type 2 diabetes and overt proteinuria (Figs 149.3, 149.4 and 149.5).

Evidence of nephrotic-range proteinuria in patients without concomitant retinopathy or arterial hypertension, or acute onset of overt proteinuria, should always raise the suspicion of associated diseases, such as lymphoproliferative disorders with Bence Jones proteinuria, systemic amyloidosis, or other glomerulopathies, including membranous nephropathy, focal segmental glomerulosclerosis, minimal change disease, and immunoglobulin A nephropathy. Since the above conditions may be treated with specific interventions (Ruggenenti et al., 2012a, 2012b), prompt histological diagnosis is warranted to establish appropriate therapy as soon as possible.

Recently a uniform classification for DN has been developed combining type 1 and type 2 DNs (Tervaert et al., 2010) (Table 149.1, Fig. 149.6); because of the important heterogeneity of lesions seen in type 2 diabetic patients, future validation of the prognostic value of this new classification system is needed.

The interstitium and the vasculature

In parallel to the glomerular lesions, tubular basement membrane thickening parallels later stages of glomerular disease (classes II–IV). With the progression of the disease, interstitial fibrosis with significant inflammatory infiltrates (T lymphocytes and macrophages) becomes more apparent and tubular atrophy is also observed (Bohle et al., 1991; Najafian et al., 2003, 2006).

Efferent arteriolar hyalinosis has been proposed as a specific lesion for DN (Stout et al., 1994), while hyalinosis of the afferent arteriole has mainly been described in other disease conditions. Arteriolar hyalinosis has been related to severity of the disease and albuminuria, although no distinction was made between efferent and afferent arteriolae (Fioretto et al., 1995; Ruggenenti et al., 1998). Severe more diffuse parenchymal vascular disease is observed mainly in more advanced cases (Bohle et al., 1991), although it has been proposed that the vascular disease process occurs since the early stages of DN (Osterby et al., 1999).

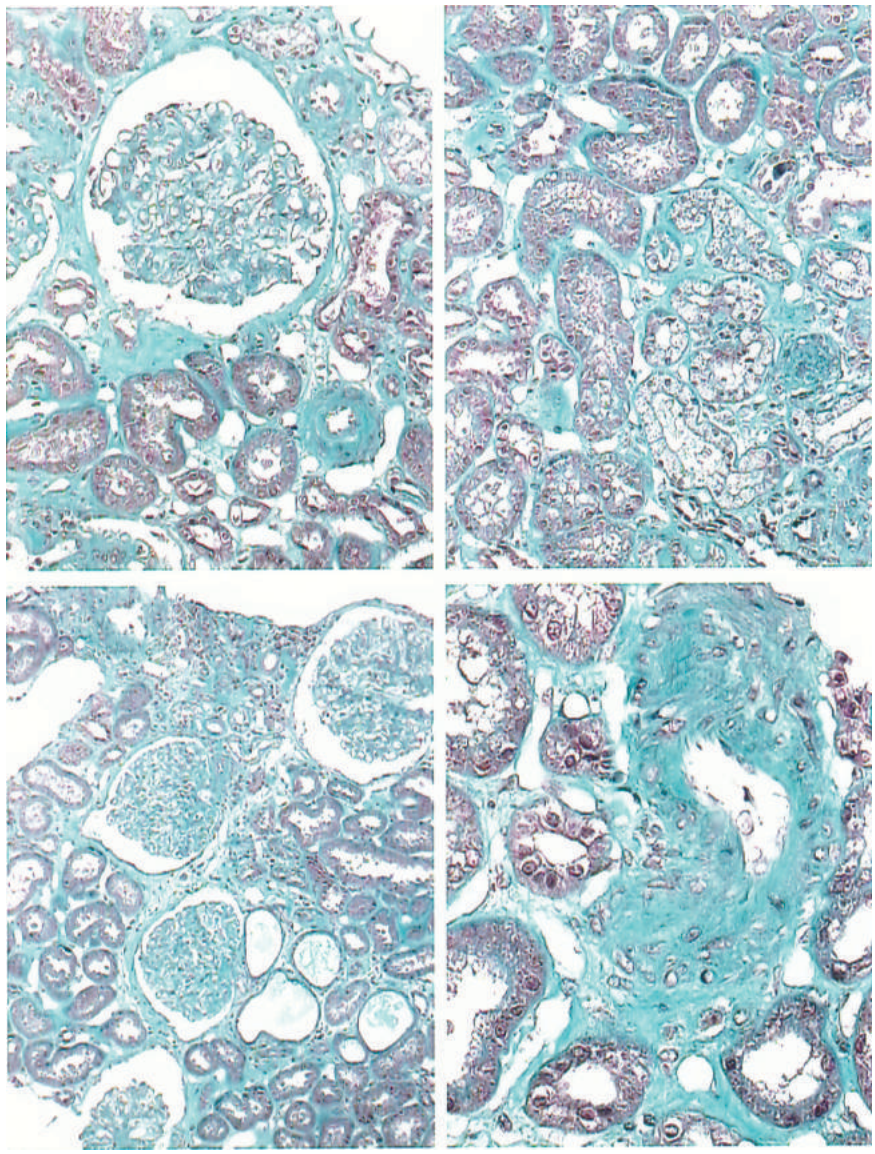

Fig. 149.1 Early diabetic nephropathy (class I): glomerular capillary wall thickening, moderate mesangial expansion and hypercellularity, mild interstitial fibrosis and moderate atherosclerosis of the vessels.

Courtesy of Franco Marchetti, Ospedale Papa Giovanni XXIII, Bergamo, Italy.

Pathophysiology of microvascular damage

Mechanisms of altered glomerular permeability

Haemodynamic and metabolic perturbations associated with the diabetic milieu are the two major determinants contributing to the development and progression of renal disease in diabetes (The Diabetes Control and Complications Trial Research Group, 1993; Hostetter, 1994; Lewis et al., 1999; Adler et al., 2000). The interplay between hypertension and renal disease is complex (Cooper, 2001; Gnudi et al., 2007): renal impairment contributes to the development and severity of hypertension, while high blood pressure accelerates the course of renal disease. Patients with diabetes and hypertension have a higher risk of ESRD than patients with hypertension or diabetes alone (Jamerson, 2005). Raised blood pressure is often paralleled by clinical albuminuria, considered the earliest manifestation of systemic microvascular damage (Deckert et al., 1989).

The interaction between hypertension and hyperglycaemia in the pathophysiology of diabetic kidney disease was initially studied by Hostetter et al. who found a hyperglycaemia-mediated altered glomerular capillaries autoregulation (Ito and Abe, 1997), that by reducing afferent and, to a much lesser degree, efferent arteriolar tone, resulted in higher glomerular hydraulic pressure, and secondary glomerular lesions (Hostetter et al., 1981, 1982).

The mechanisms at the basis of hyperglycaemia-mediated disruption of capillary vasoregulation are complex and yet not fully elucidated. Hyperglycaemia-mediated increase in vascular nitric oxide (NO) (De Vriese et al., 2001a) and transforming growth factor beta-1 (TGF-β1) (Kagami et al., 1994; Raij, 2005) (through the production of reactive oxygen species (Sharma et al., 2005)) have been implicated in vasodilation of both afferent and efferent glomerular arteriolae. Hyperglycaemia also activates the local tissue renin–angiotensin–aldosterone system (RAAS) (Anderson and

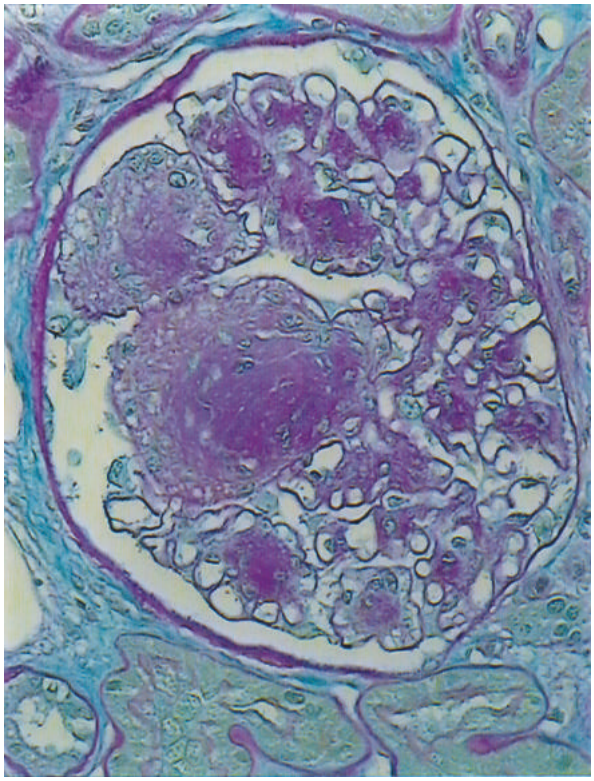

Fig. 149.2 Glomerulus with Kimmelstiel–Wilson lesion. Nodular mesangial sclerosis (pink hyaline material) with accentuated, lobular-appearing glomerulus. Although classically described in association with diabetes, the lesion is not pathognomonic for diabetic nephropathy and is also found in a number of glomerulopathies, including immunotactoid glomerulonephritis, amyloidosis, fibrillary glomerulonephritis, light or heavy chain deposition disease, cryoglobulinaemic glomerulonephritis, and idiopathic nodular glomerulosclerosis. Congo red staining and immunofluorescence may help in ruling out diagnosis. Courtesy of Franco Marchetti, Ospedale Papa Giovanni XXIII, Bergamo, Italy.

Vora, 1995) with local excess production of angiotensin II. In diabetes, the documented higher sensitivity of the efferent (versus the afferent) glomerular arteriole to the vasoconstrictive action of angiotensin II, contributes to the imbalance in arteriolar tone which then results in higher glomerular capillary pressure (Raij, 1995; Maddox and Brenner, 2000). As a result, in diabetes, a disproportionate systemic pressure is transmitted to the glomerular circulation resulting in glomerular cell mechanical elongation and activation of the cellular mechanisms that lead to glomerular damage (Arima and Ito, 2003).

Similarly in experimental animal models of glomerular hypertension, such as the diabetic (or uninephrectomized) spontaneously hypertensive rat, haemodynamic perturbations result in earlier and significant increases in albuminuria, increased TGF-β1, mesangial expansion, and glomerulosclerosis (Cooper, 2001). To link, at the molecular level, metabolic and haemodynamic perturbations, investigators have focused their attention on GLUT-1 facilitative glucose transporter (Thorens and Mueckler, 2010). GLUT-1 expression levels in mesangial cells have been closely related with extracellular matrix protein expression (Heilig et al., 1995, 2001), and GLUT-1 protein upregulation has been proposed as one of the mediators of metabolic–haemodynamic interaction in diabetic glomerulopathy (Gnudi et al., 2003, 2007).

The excess of local angiotensin II in DN is a major direct stimulus for upregulation of TGF-β1 (Wolf and Ziyadeh, 1999; Daniels et al., 2000; Weigert et al., 2002), one of the major prosclerotic cytokines (Ziyadeh and Han, 1997), which in turn stimulates the expression of different cytokines such as VEGF-A, monocyte chemoattractant protein-1 (MCP-1) (Wolf and Ziyadeh, 2007), TGF-β1 itself (Ziyadeh, 2004), and connective tissue growth factor (CTGF) (Riser and Cortes, 2001; Burns et al., 2006). The excessive production of TGF-β1 and the activation of TGF-β1-mediated pathways are involved in the steps that progressively lead to glomerulosclerosis and renal interstitial fibrosis (Gruden et al., 2000; Chen et al., 2005). Importantly, angiotensin II and TGF-β1 stimulate NADPH oxidase (Onozato et al., 2002), leading to excess reactive oxygen species accumulation, and stimulate GLUT-1 protein expression and glucose uptake in mesangial cells (Gnudi et al., 2007). The excess cellular glucose uptake, driven by the ambient elevated circulating glucose levels, results in activation of different intracellular metabolic pathways such as the polyol and hexosamine pathway, increased production of advanced glycation end-products, activation of protein kinase C (PKC) and p38 mitogen activated protein kinase (MAPK), and increase in oxidative stress (Brownlee, 2001, 2005), pathways linked to upregulation of mediators of glomerular damage such as angiotensin II, VEGF-A, and TGF-β1.

The endothelium seems to play a central role in the pathophysiology of diabetic glomerulopathy. Endothelial dysfunction has been shown to precede increased vascular permeability and albuminuria (Lim et al., 1999; Persson et al., 2008), which, as suggested in the 'Steno hypothesis', could represent a common pathogenetic mechanism for renal and extrarenal chronic vascular diabetic complications (Deckert et al., 1989; Stehouwer, 2004). Markers of endothelial dysfunction such as soluble intercellular and vascular adhesion molecules, von Willebrand factor, and altered microvascular reactivity can be observed in patients with type 2 diabetes before the onset of albuminuria (Lim et al., 1999; Persson et al., 2008).

Impairment in number and function of endothelial progenitor cells, involved in neovasculogenesis, endothelium repair, and maintenance of vascular homeostasis, has been proposed as a pathogenic mechanism for vascular disease in diabetes, highlighting the important link between endothelial dysfunction and DN (Fadini et al., 2006; Hohenstein et al., 2010). Studies within populations of patients with type 1 or type 2 diabetes have demonstrated a reduced number and impaired function of circulating vascular progenitor cells in patients with microalbuminuria (Makino et al., 2009; Dessapt et al., 2010).

In diabetes, glomerular endothelial cell injury with loss of glycocalyx and cell apoptosis has been proposed as one of the mechanisms of diabetic glomerulopathy (Satchell and Tooke, 2008). The glycocalyx, composed of heparan sulphates, hyaluronic acid, sialoprotein, and proteoglycans (Reitsma et al., 2007; Weinbaum et al., 2007), is a aqueous extracellular layer that covers the glomerular capillary lumen side, and changes in glycocalyx have been linked to changes in endothelial and glomerular vascular function and permeability (Haraldsson et al., 2008; Satchell and Tooke, 2008).

Diabetes-mediated insults to the endothelium derive mainly from direct *noxae* such as raised blood glucose level, advanced glycation end-products, increased oxidative stress, and haemodynamic perturbations due to local RAAS activation and secondary increase in glomerular capillary pressure (Gnudi et al., 2007). These perturbations also affect other glomerular cells, such as podocytes

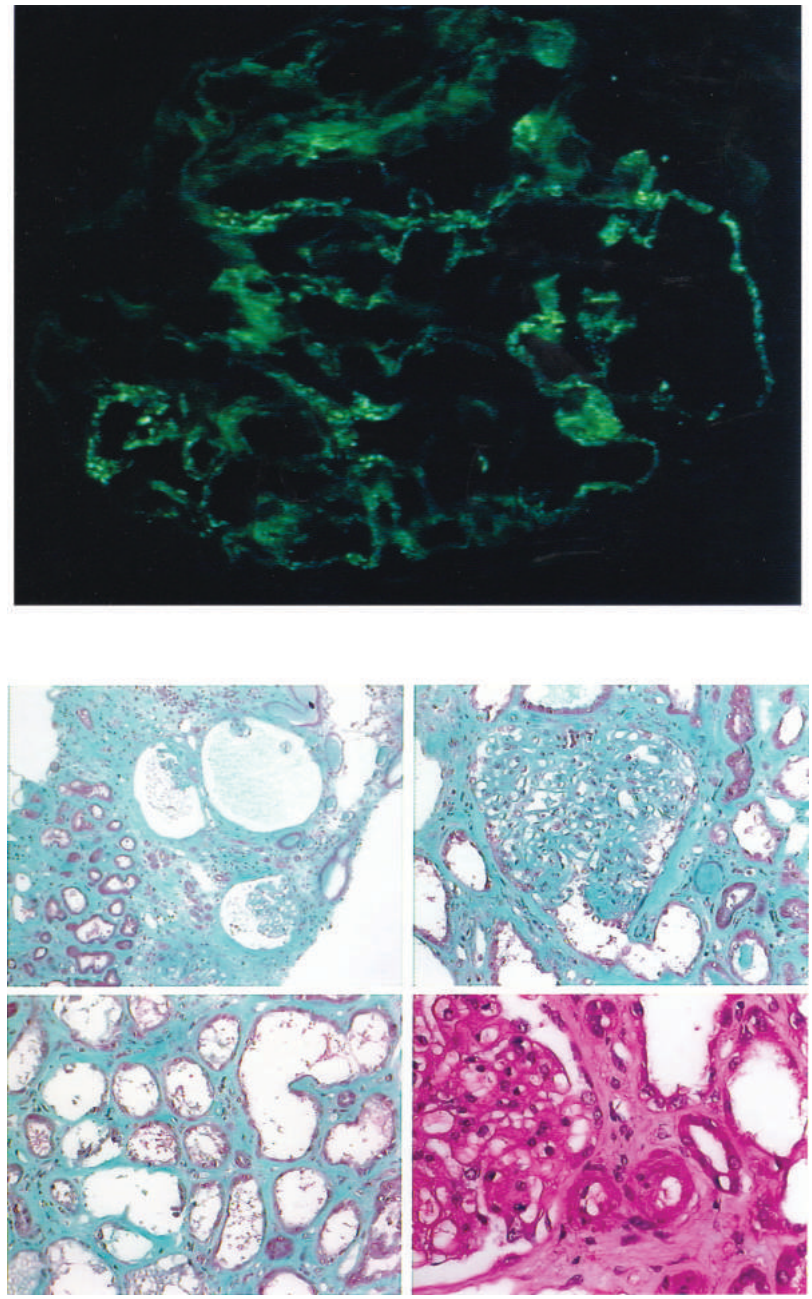

Fig. 149.3 Moderate-severe diabetic nephropathy with superimposed membranous nephropathy. Light microscopy (lower panel) shows diffuse glomerulosclerosis, severe interstitial fibrosis, diffuse glomerular capillary wall thickening, and tubular degeneration and necrosis. Immunofluorescence (upper panel) shows fine parietal granular deposits of IgG and C3.
Courtesy of Franco Marchetti, Ospedale Papa Giovanni XXIII, Bergamo, Italy.

and mesangial cells, resulting in podocyte effacement, apoptosis, podocyte loss in the urine (Pagtalunan et al., 1997), and mesangiolysis, affecting not only the anatomical structure of the glomerular filtration barrier but also altering the autocrine/paracrine actions of secreted cytokines and vascular growth factors (e.g. TGF-β1, VEGF-A, and angiopoietins) by these cells, resulting in alteration of the 'local glomerular cells network' crucial in the maintenance of the normal function of the glomerular filtration barrier.

Specifically the retrograde flow of VEGF-A and possibly other podocyte-secreted cytokines such as angiopoietins (Woolf et al., 2009) appear to be very important in the physiology of the glomerular filtration barrier; the subpodocyte space, covering 60–70% of the entire filtering surface of the capillary walls, has been implicated in the regulation of the movements of particles across the filtration barrier. The subpodocyte space comprises an area between the podocyte cell body and the GBM (Neal et al., 2005) where the filtrate accumulates before gaining access to Bowman's space via small areas directly connected to the urinary space. This structure is believed to confer a degree of resistance to filtration, that through changes in pressure of the subpodocyte space (likely modulated by a finely regulated contraction of the podocytes) favours the movement of proteins/cytokines against the

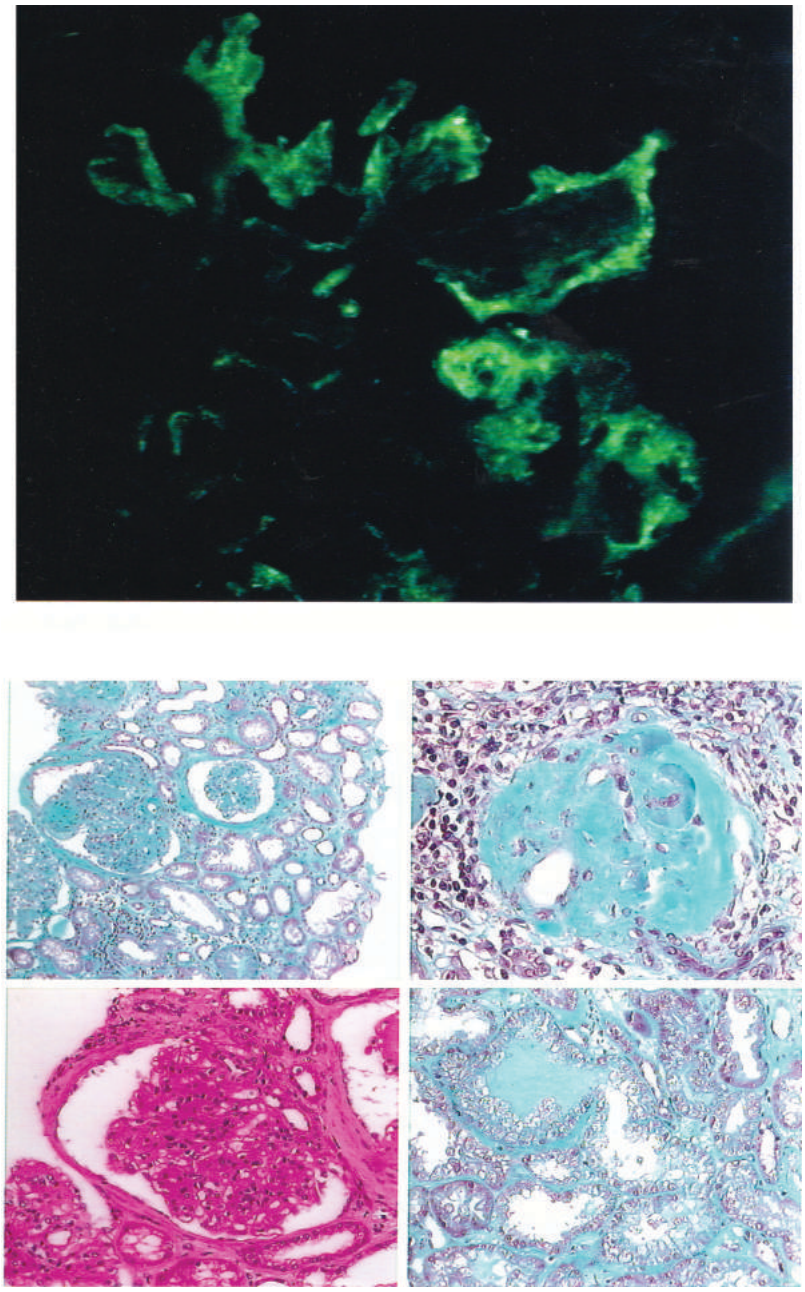

Fig. 149.4 Severe diabetic nephropathy and superimposed hepatitis C virus-related glomerulonephritis. Light microscopy (lower panel) shows severe nodular and diffuse glomerulosclerosis, mild mesangial and endocapillary hypercellularity, moderate to severe interstitial fibrosis, diffuse tubular vacuolization, and severe atherosclerosis. Immunofluorescence (upper panel) shows prevalently parietal granular deposits of IgM and C3.
Courtesy of Franco Marchetti, Ospedale Papa Giovanni XXIII, Bergamo, Italy.

net filtration flow allowing podocyte-secreted molecules to interact with the endothelial/mesangial glomerular cellular network (Salmon et al., 2007, 2009a).

In this regard, in experimental animal models of diabetes, the podocyte appears, as the glomerular endothelial cells, to retain a key role in preserving glomerular capillary integrity, regulating synthesis of extracellular matrix protein in the GBM, and maintaining restriction to protein filtration via the interdigitation of its foot processes (Faul et al., 2007). In various experimental animal models of diabetes, podocyte loss is followed by remaining podocytes trying to cover a larger GBM surface area; this is followed by podocyte foot process widening, proteinuria, and subsequently glomerulosclerosis (Fries et al., 1989; Nagata and Kriz, 1992; Gassler et al., 2001; Hoshi et al., 2002). Specifically it has been observed that early glomerular changes in the ZDF-fa/fa rat and Goto Kakizaki rats, animal models for type 2 diabetes, are characterized by podocyte injury without clear evidence of mesangial expansion (Coimbra et al., 2000; Gassler et al., 2001; Hoshi et al., 2002).

In humans, it has been difficult to stage the morphological changes that occur during the early phases of diabetic glomerulopathy, but the loss of podocytes, which might expose an area of bare GBM, would represent a potential 'trigger' for glomerular injury

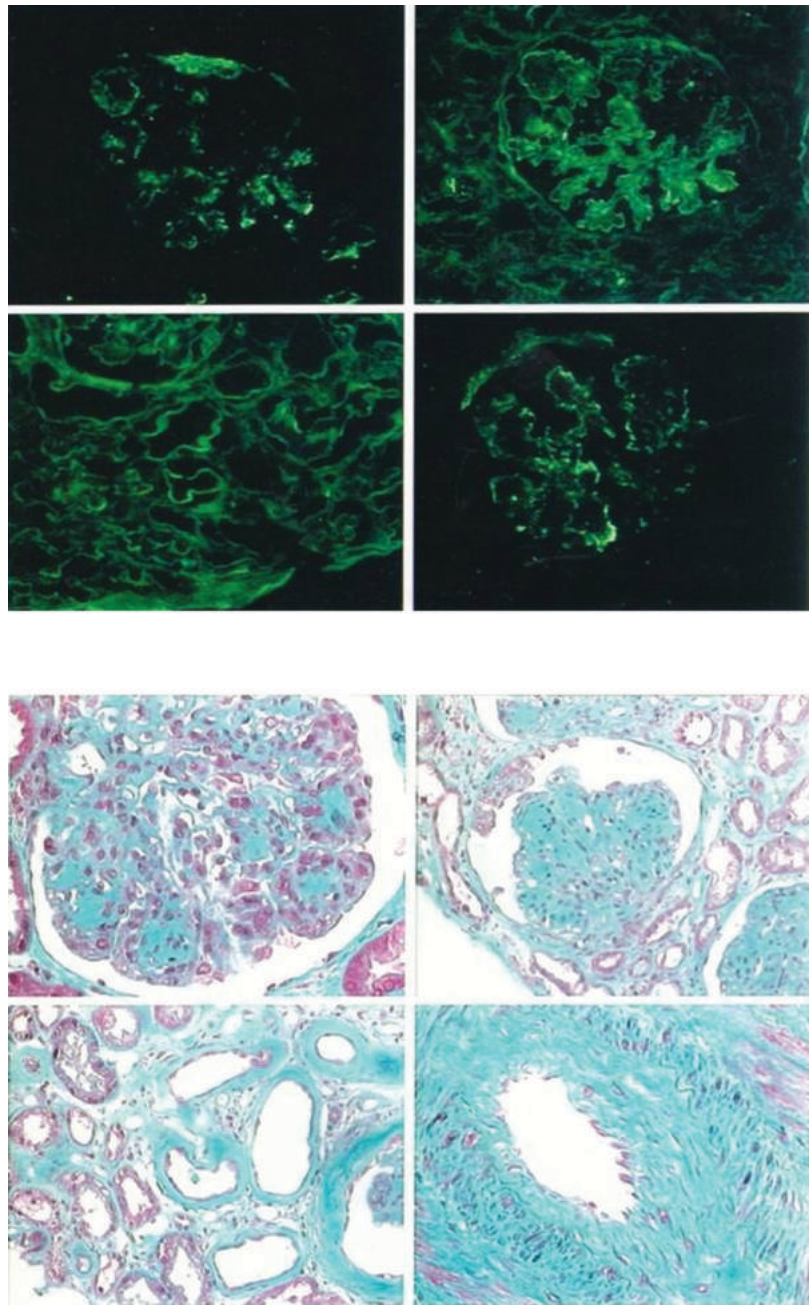

Fig. 149.5 Severe diabetic nephropathy and superimposed cryoglobulinaemic glomerulonephritis. Light microscopy (lower panel) shows a severe case of nodular glomerulosclerosis, mesangial hypercellularity, glomerular capillary wall thickening, endocapillary proliferation with capillary lumen narrowing, and moderate interstitial fibrosis with focal tubular atrophy, and moderate-severe atherosclerosis. Immunofluorescence (upper panel) shows glomerular and tubular deposits of kappa and lambda light chains, IgM, IgG, and IgA.
Courtesy of Franco Marchetti, Ospedale Papa Giovanni XXIII, Bergamo, Italy.

(Kriz et al., 1994); this is likely to occur in parallel with mesangial expansion and glomerular extracellular matrix deposition which closely relate to albuminuria (Mauer et al., 1984). Along this line, a recent study highlighted the key role of podocyte detachment and loss of normal endothelial cell fenestration in 37 patients with type 2 diabetes undergoing renal biopsy. The percentage of podocyte detachment correlated positively with urinary albumin excretion and negatively with the number of podocytes per glomerulus, while the percentage of endothelial cell fenestrations associated negatively with GBM width, fractional interstitial, and mesangial area, and positively with filtration surface area density and GFR. Increasing podocyte detachment was associated with decreased permselectivity of the glomerulus and progressive albuminuria, while loss of endothelial cell fenestration was associated with GFR decline (Weil et al., 2012).

Alteration in VEGF-A glomerular expression has been demonstrated to be crucial in the pathophysiology of diabetic glomerular disease. Development of proteinuria and glomerular damage

Table 149.1 Glomerular classification of diabetic nephropathy

Class	Description	Inclusion criteria
I	Mild or non-specific LM changes and EM-proven GBM thickening	Biopsy does not meet any of the criteria mentioned below for class II, III, or IV GBM > 395 nm in female and > 430 nm in male individuals 9 years of age and older
IIa	Mild mesangial expansion	Biopsy does not meet criteria for class III or IV Mild mesangial expansion in > 25% of the observed mesangium
IIb	Severe mesangial expansion	Biopsy does not meet criteria for class III or IV Severe mesangial expansion in > 25% of the observed mesangium
III	Nodular sclerosis (Kimmelstiel–Wilson lesion)	Biopsy does not meet criteria for class IV At least one convincing Kimmelstiel–Wilson lesion
IV	Advanced diabetic glomerulosclerosis	Global glomerular sclerosis in > 50% of glomeruli Lesions from classes I through III

EM = electron microscopy; GBM = glomerular basement membrane; LM = light microscopy.

From Tervaert, T. W., Mooyaart, A. L., Amann, K., *et al.* (2010). Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol*, 21(4), 556–63.

is associated with VEGF-A overexpression in the podocytes, and intervention aiming at inhibiting VEGF-A in animal experimental setting has demonstrated that administration of VEGF inhibitors, such as antibodies or chemicals, ameliorates diabetes-mediated proteinuria and glomerular damage (De Vriese et al., 2001b; Flyvbjerg et al., 2002; Sung et al., 2006; Ku et al., 2008). The contention of paracrine/autocrine action of podocyte-secreted VEGF is supported by the presence of VEGF receptors in endothelial cells, mesangial cells (Cooper et al., 1999; Thomas et al., 2000; Sison et al., 2010), and podocytes (Ku et al., 2008; Veron et al. 2010). Along similar lines the induction of primary podocyte-specific VEGF-A overexpression in adult mice results in glomerular filtration barrier structural and functional abnormalities similar to those present in murine diabetic glomerulopathy (Veron et al., 2010).

In experimental animal model of diabetes, the interaction of VEGF-A with NO plays an important role in the alteration of endothelial function (Nakagawa, 2007). In diabetes, glomerular disease, decrease in endothelial NO production secondary to endothelial nitric oxide synthase (eNOS) uncoupling (Schulz et al., 2008), or downregulation of eNOS expression appear to play an important pathogenic role (Zhao et al., 2006; Wang et al., 2011). Paradoxically in type 2 diabetic patients, increased eNOS expression by the renal endothelium has been shown to relate to vascular lesions and the degree of proteinuria (Hohenstein et al., 2008). Importantly in diabetes, impaired eNOS activation and uncoupling results in a fall of NO availability (Nakagawa et al., 2007) and increased reactive oxygen species production, key players in the pathogenesis of microvascular complications (Santilli et al., 2004).

Importantly, ambient levels of VEGF-A affects the actions of angiopoietin (Angpt)-1 and -2 on the vasculature. Angiopoietins are vascular growth factors involved in angiogenesis and vasculogenesis (Hanahan, 1997). Two major isoforms have been described, Angpt-1 and Angpt-2, both ligands for the Tie-2 receptor, expressed primarily on endothelial cells and podocytes (Satchell et al., 2002; Woolf et al., 2009). In physiology, Angpt-1 is expressed in podocytes while Angpt-2 is only expressed during glomerular development mainly by immature mesangial cells (Woolf et al., 2009). Angpt-1, the major physiological ligand for Tie-2, promotes endothelial cell survival, stabilization of supporting perivascular cells, and inhibition of endothelial permeability (Satchell et al., 2004). Angpt-2 is considered to be a natural antagonist of Angpt-1 by virtue of its ability to competitively inhibit binding of Angpt-1 to Tie-2, hence

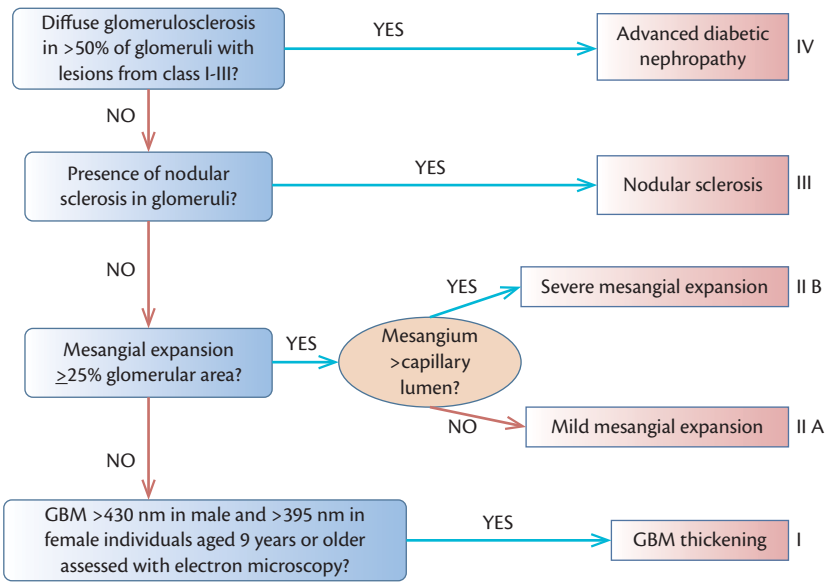

Fig. 149.6 Flow chart for classifying diabetic nephropathy.

Modified from Tervaert, T. W., Mooyaart, A. L., Amann, K., *et al.* (2010). Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol*, 21(4), 556–63.

reducing Tie-2 activation and signalling. The angiopoietin/Tie-2 receptor system has been implicated in the pathogenesis of diabetic glomerulopathy (Yamamoto et al., 2004; Lim et al., 2005; Rizkalla et al., 2005; Ichinose et al., 2006) and in type 2 diabetic patients, circulating and urinary Angpt-2 levels are elevated (Lim et al., 2005). These changes lead to glomerular neovascularization which has been shown to be associated with glomerular hypertrophy; inhibition of diabetes-mediated angiogenesis results in reduced GBM thickening, mesangial expansion, and TGF-β1 expression in diabetic mice (Yamamoto et al., 2004; Ichinose et al., 2005, 2006).

VEGF-A has been partly implicated in the regulation of the glycocalyx structure (Fu and Shen, 2003) and recent work has shown that Angpt-1 increases the depth of endothelial glycocalyx in intact microvessels and reduces glomerular capillary permeability (Salmon et al., 2009b). Angpt-1 and Angpt-2 levels are deregulated (often with Angpt-2 > Angpt-1) in conditions of altered glomerular permeability (albuminuria) such as diabetic glomerulopathy.

Transgenic mice with podocyte-specific inducible overexpression of Angpt-2 show a significant increase in albuminuria and glomerular endothelial apoptosis (Davis et al., 2007). Similarly, when Angpt-1 was ablated specifically from podocytes or mesangial cells, animals showed accelerated diabetes-mediated glomerular damage suggesting that glomerular Angpt-1 expression may confer protection against microvascular glomerular injury (Jeansson et al., 2011). In addition, podocyte-specific Angpt-1 repletion in experimental animal model of diabetic kidney disease ameliorates albuminuria (Dessapt-Baradez et al., 2014).

Mechanisms of tubular disease and interstitial fibrosis

Similar players are involved in the mechanisms contributing to interstitial fibrosis and tubular atrophy. Similarly to the glomerulus, metabolic insults (hyperglycaemia and advanced glycation end-products), hypoxia, inflammation, oxidative stress, and nitrosative stress have been involved in tubulointerstitial damage (Vallon, 2011). It is now recognized that tubules play an important role in the pathogenesis of DN and evidence exist that renal function correlates also with tubulointerstitial changes (Gilbert and Cooper et al., 1999).

Tubular proteinuria may precede microalbuminuria (Ginevri et al., 1993), suggesting that tubular damage occurs early in the course of the disease. Indeed, urinary liver-type fatty acid-binding protein (L-FABP), a marker of tubulointerstitial damage, correlates closely with renal dysfunction in diabetic subjects (Nakamura et al., 2005; Suzuki et al., 2005), even in the absence of microalbuminuria (Cabre et al., 2008).

Hyperglycaemia affects the tubular structures both from the base-lateral side and at the same time determines an increase in glucose filtration in the glomeruli that results in an increase in tubular glucose load and exposure. Further, the excess of renally filtered glucose in diabetes is paralleled by upregulation, in rodents and humans, of the low-affinity/high-capacity Na^+-glucose co-transporter SGLT2 (mainly localized in the proximal tubuli) (Rahmoune et al., 2005; Tabatabai et al., 2009) known to represent the major player in glucose reabsorption in the nephron (Hummel et al., 2011). The upregulation of SGLT2 is paralleled by activation of the local angiotensin II system and growth factors (e.g. CTGF, TGF-β1) which in turn will favour early tubular cell proliferation, followed by tubular hypertrophy and senescence and increased deposition of extracellular matrix (Wolf and Ziyadeh, 1999; Vallon et al., 2011).

Studies in normoalbuminuric patients with type 1 diabetes mellitus found a significant correlation between glomerular hyperfiltration and fractional proximal reabsorption of sodium (Vervoort et al., 2005), and the risk of progression to microalbuminuria, along with a faster decline in GFR, is higher in patients with increased kidney volume (Zerbini et al., 2006). This work suggests that tubular hypertrophy and glucose/Na^+ hyper-reabsorption contribute to glomerular hyperfiltration in the initial stages of DN. Hyperfiltration, in turn, participates with the glomerular capillary dysregulation to glomerular hypertension with associated increased filtration of proteins to tubular lumen, resulting in tubulointerstitial inflammation and fibrosis, a cycle culminating in ESRD (Remuzzi and Bertani, 1998).

Studies in Pima Indians show that, at early stages of diabetic renal disease, normal albumin excretion or microalbuminuria associate with elevation in GFR, while transition to macroalbuminuria heralds a progressive decline in glomerular filtration (Nelson et al., 1996), that is sustained by enhanced glomerular protein ultrafiltration with glomerular and tubular overload, chronic inflammation, and further acceleration of glomerular and tubulointerstitial damage, progressive glomerulosclerosis, and kidney failure (Remuzzi and Remuzzi, 1994, 1998; Lemley et al., 2000). Thus, in advanced stages of diabetic kidney disease, albumin and, even more, other plasma proteins, complement components, and growth factors ultrafiltrated in excess through the damaged glomerular barrier play a central role in the progression of diabetic renal disease. This may explain why interventions that reduce protein traffic and, consequently, proteinuria are invariably renoprotective in this context (Remuzzi et al., 2006; Ruggenenti and Remuzzi, 2006; Ruggenenti et al., 2012f).

Clinical presentation

The evolution of DN proceeds through several distinct but interconnected phases, which ultimately lead to ESRD (Table 149.2).

The earliest clinical manifestation of diabetic renal injury is the excretion of small amounts of albumin in the urine, the so-called microalbuminuric range (Viberti et al., 1982; Karalliedde and Viberti, 2004). Now we know that even smaller amounts of urinary albumin strongly predict the onset of microalbuminuria in individuals with type 2 diabetes (Ruggenenti et al., 2004; Ruggenenti and Remuzzi, 2006), on the other hand, prospective studies conducted in patients with type 1 diabetes have shown that a fall in renal function might precede, in some instance, the onset of albuminuria (Caramori et al., 2003; Perkins et al., 2007; Premaratne et al., 2008).

Because of the natural history of type 2 diabetes where approximately 18% of patients present at diagnosis with albuminuria (Remuzzi et al., 2002b), most studies on early phases of diabetic kidney disease have been conducted in type 1 diabetic patients.

Early renal abnormalities: hyperfiltration

Seminal observations by Mogensen and co-workers underscored that early phases of diabetic renal disease are characterized by an elevation of renal plasma flow of approximately 30%, paralleled by an increase in GFR (Mogensen, 1971, 1986). Increased GFR is, at times, paralleled by microalbuminuria that is often reversed by better glycaemic control, even at a time when systemic arterial

Table 149.2 Classification of stages of diabetic nephropathy, modified according to KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease (KDIGO, 2013)

Persistent albuminuria categories	24-hour urine collection: albumin excretion rate (mcg/min mg/24 h)	Spot urine collection: albumin:creatinine ratio (mg/mmol mg/g)	Glomerular filtration rate	Blood pressure
Normal to mildly increased (A1)	<20 <30	<3 <30	Normal or high (G1)	Increasing
Moderately increased (A2)	20–200 30–299	3–30 30–300	Normal or high (G1)	Rising
Severely increased (A3)	>200 ≥300	>30 >300	Decreased (G2, G3a, G3b, G4, G5)	Elevated

pressures are unequivocally normal (Stephenson and Fuller, 1994). Previous studies and a recent meta-analysis have suggested that the glomerular hyperfiltration seen in patients with diabetes is a significant risk factor for progression to microalbuminuria and advanced diabetic kidney disease (Mogensen, 1986; Magee et al., 2009) although these findings were not confirmed in other series (Nelson et al., 1996; Yip et al., 1996; Chaiken et al., 1998; Zerbini et al., 2006). The above inconsistencies were most likely explained by small sample size and heterogeneity of the above studies that enhanced random data fluctuations. The pathogenic role of glomerular hyperfiltration was recently confirmed by a cross-sectional and longitudinal analysis of 600 type 2 diabetes patients who had their GFR prospectively monitored by the iohexol plasma clearance technique. Data showed that, over a median follow-up period of 4 years, hyperfiltration was an independent risk factor for accelerated renal function loss and for onset and progression of DN, in particular in those patients with persistent hyperfiltration despite intensified metabolic and blood pressure control (Ruggenenti et al., 2012c). As suggested by studies on filtration fractions (GFR/renal plasma flow), an indirect measure of glomerular capillary pressure, the increased risk seen in patients with glomerular hyperfiltration is most likely explained by a concomitant increase in glomerular pressure, which in turn contributes to progressive structural damage. Thus even at 'normal' systemic arterial pressures, in the presence of hyperglycaemia, glomerular capillary pressure is elevated and may synergize with the metabolic perturbation in the disease process (Poulsen et al., 1994; Lurbe et al., 2002).

Hyperglycaemia-induced hyperfiltration may occur even in advanced stages of the disease without major changes in renal plasma flow, likely via changes in glomerular filtration barrier permeability, a mechanism that may contribute to the progression of DN even in patients with renal insufficiency (Remuzzi et al., 1990).

In addition, the progressive loss of functioning nephrons and the compensatory hyperfiltration of remnant nephrons also contribute to the progression of chronic kidney damage in diabetic renal disease, as also seen in all progressive nephropathies (Anderson and Brenner, 1986).

Ethnicity is an important factor, and diabetic patients with Asian and African origin often predisposed to raised blood pressure levels, are at higher risk of kidney disease (Powers and Wallin, 1998; Crook and Patel, 2004). Phenotypically, these populations seem to be characterized by higher salt sensitivity, and a salt-rich diet triggers increased RAAS activity leading to increased glomerular capillary pressure (Weir, 1998). Higher salt sensitivity is also driven by insulin resistance in patients with type 1 and type 2 diabetes (Trevisan et al., 1998; Vedovato et al., 2004), and faster and progressive diabetic kidney damage has been associated with insulin resistance (Groop et al., 1993; Yip et al., 1993).

Progression of diabetic nephropathy

The phases of DN have been historically depicted as progression through different stages of albuminuria: normo- to microalbuminuria, micro- to macroalbuminuria, and macroalbuminuria to renal failure.

Although the terms normoalbuminuria, microalbuminuria, and macroalbuminuria (clinical albuminuria) describe different categories of UAER it is important to remember that they are part of a continuum in the relationship between albumin excretion and cardiorenal risk (Gerstein et al., 2001; De Zeeuw, 2004; Ruggenenti and Remuzzi, 2006; Ruggenenti et al., 2012d). Even in patients with type 2 diabetes and normoalbuminuria, any degree of measurable albuminuria bears significant cardiovascular risk; this association is lost when the risk is uniformly prevented at any level of albuminuria with RAAS inhibition therapy (Ruggenenti et al., 2012d). Further post hoc analyses of randomized trials in high-risk subjects and community-based cohort studies showed that incremental increases in albuminuria within the 'normal' range carry an increased risk of nephropathy or cardiovascular events (Gerstein et al., 2001; Wachtell et al., 2003; Klausen et al., 2004).

Recently, efforts have been conducted to better define and classify CKD by the Kidney Disease Improving Global Outcomes (KDIGO) initiative (Levey et al., 2011; KDIGO, 2013). This initiative combines albuminuria and GFR, trying to maximize the expected patients' outcome in terms of cardiovascular risk and renal outcome. The strong association existing between GFR and the thresholds of albuminuria will set a better risk prediction instrument in terms of cardiovascular-renal risk. Future research and clinical practice guideline will have to recommend specific, possibly more stringent guidelines specifically for diabetes.

In the new guideline, the Work Group has recommended the classification of albuminuria into only three categories: normal to mildly increased (normoalbuminuria or high-normal albuminuria), moderately increased (microalbuminuria), and severely increased (macroalbuminuria). Clearly, this will require a formal education programme and review of existing guidelines in other disciplines so that consistency of terminology and understanding of the changes are universal. For this reason, both nomenclatures will be used in the present chapter.

Moderately increased albuminuria (microalbuminuria)

In normal physiology, nearly the entire small amount of albumin filtered by the glomerulus is reabsorbed in the proximal tubules of the kidney. This reabsorptive process is at near maximal capacity so that moderate increases in filtered albumin result in elevated albumin excretion in the urine. The reabsorptive process is proportional to the amount of albumin filtered through the glomerular filter, but when the amount of filtered albumin exceeds the maximal capacity of tubular albumin reabsorption, the proportion of albumin excreted in the urine increases. In patients with clinical albuminuria, albumin represents approximately 50% of total urinary protein. Most patients will exhibit microalbuminuria (UAER ranging between 20 and 200 micrograms/min) well before the onset of overt clinical albuminuria (Table 149.2). It is accepted that the rise in UAER seen in patients with microalbuminuria reflects an increased transglomerular flux of albumin as a consequence of an increased transglomerular pressure gradient and likely alteration in the endothelial layer and loss in negative charge of the glomerular filtration barrier (Haraldsson et al., 2008). As the disease progresses, an increase in glomerular membrane pore size with disruption of the three layers (endothelial cells, GBM, and podocytes) of the glomerular filter, also contributes to albuminuria (Torffvit et al., 1999; Oberbauer et al., 2001). This paradigm has recently been challenged with the suggestion that the glomerulus is physiologically significantly less restrictive to the filtration of plasma albumin and that it is tubular damage which largely accounts for the increased albumin in the urine (Russo et al., 2009), but this concept has been heavily disputed (Haraldsson et al., 2008; Tanner, 2009).

Without intervention, microalbuminuria progresses towards clinical albuminuria over approximately 10–15 years of disease (Karalliedde and Viberti, 2004). In healthy adults, the normal UAER ranges between 1.5 and 20 micrograms/min with median value around 6.5 micrograms/min (Karalliedde and Viberti, 2004). The average day-to-day variation of UAER is about 40% and similar in diabetic and non-diabetic individuals. In view of this high biological variability the diagnosis of microalbuminuria should ideally be made from the calculation of the median value of at least three timed, non-consecutive urine collections over a short time period. In the day-to-day clinic setting, the calculation of the albumin-to-creatinine ratio (ACR) in an early morning urine sample has replaced the measurement of albumin on timed urine collection and has proven to be of acceptable accuracy (Karalliedde and Viberti, 2004). ACR correlates closely with UAER, and recently has been proposed as an important marker for prediction of renal events in diabetic patients (Lambers Heerspink et al., 2010).

The exact significance of microalbuminuria in patients with short-term duration of diabetes is unclear, and the observed changes in microalbuminuria are not always associated with a commensurate alteration/decline in renal function, especially in the early phase of renal disease in diabetes (Jerums et al., 2008; Karalliedde and Viberti, 2010).

Approximately 2% of patients with type 1 diabetes with normal UAER develop albuminuria per year (Chaturvedi et al., 2001), and rate of transition from normoalbuminuria to microalbuminuria is around 4% per annum in patients with type 2 diabetes (Nelson et al., 1995; Gall et al., 1997; Forsblom et al., 1998). Factors favouring its progression include baseline UAER, poor glycaemic control, blood pressure, especially an increase of systolic blood pressure during sleep, presence of retinopathy, smoking, and dyslipidaemia (Nelson et al., 1993, 1997; Gall et al., 1997; Ravid et al., 1998; Hovind et al., 2004).

Morphological studies have clearly shown that structural abnormalities and lesions such as increased mesangial fractional volume and decreased filtration surface area are more pronounced and advanced in patients with microalbuminuria. Once microalbuminuria is established the UAER tends to rise over time. However, recent studies in patient with type 1 diabetes suggest that in approximately 30% of patients UAER reverts back towards the normal range (< 30 micrograms/min), in 50% it remains in the microalbuminuric range, and in around 20% microalbuminuria progresses towards overt albuminuria over 5–10 years (Almdal et al., 1994; Microalbuminuria Collaborative Study Group, United Kingdom, 1995; The DCCT Research Group, 1995; Hovind et al., 2004). This change in the natural history of microalbuminuria most likely reflects changes and advances in medical care with ever more stringent glycaemic, lipid, and blood pressure control as well as the widespread use in recent years of agents such as angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) (Maione et al., 2011). Even in patients with persistent normoalbuminuria, transient increases in UAER may be detected during periods of poor glycaemic control and intercurrent illnesses. Other conditions recognized as potential causes of a transient increase in urine protein are exercise, standing position, increased diuresis, and protein-rich meals.

The presence of microalbuminuria and the rate of its progression to macroalbuminuria is consistently associated with higher blood pressure independent of age, duration of diabetes, gender, or body mass index and this increase in pressure values of about 10–15% above that of normoalbuminuric patients occurs initially within the so-called 'normal' blood pressure range. Higher blood pressure values may indeed precede and predict the development of microalbuminuria suggesting that elevations in blood pressure and UAER initially through the normal range of values may represent concomitant manifestations of a common process leading to renal injury (The DCCT Research Group, 1995).

Severely increased albuminuria (macroalbuminuria—overt nephropathy)

In those patients who develop clinical albuminuria (UAER > 300 mg/day), GFR gradually declines in a linear fashion at variable rates (average 4.5 mL/min/year) depending on control of promoters of progression (metabolic, haemodynamic control), degree of albuminuria, and individual response to treatment (Hovind et al., 2004; Ruggenenti et al., 2008a). Blood pressure is probably the most important variable in the rate of progression from microalbuminuria to proteinuria (Microalbuminuria Collaborative Study Group, United Kingdom, 1995); patients will progress through phases of intermittent proteinuria and will then reach a more sustained proteinuria in the nephrotic range (Hasslacher et al., 1985, 1993).

In patients with macroalbuminuria, protein trafficking plays an additional pathogenetic role in the progression of renal disease and this may explain the acceleration in renal function loss that accompanies transition from micro- to macroalbuminuria (Ruggenenti et al., 2012f). There is a biologic gradient between the degrees of

clinical albuminuria and the progression towards ESRD. Patients with type 2 diabetes with albuminuria ≥ 3000 mg/g retain an eight-fold higher risk for progression to ESRD when compared with patients with lower albuminuria (< 1500 mg/g) (de Zeeuw et al., 2004); quantitatively every 50% reduction in albuminuria translates in a risk reduction for ESRD of around 40% (de Zeeuw et al., 2004). Lower levels of albuminuria, in the microalbuminuria and normoalbuminuria range, do not appear to predict renal disease progression and evidence is still inconclusive or not available (Perkins et al., 2007).

Although variations in fall in GFR exist between patients, the overall rate of fall remains relatively constant for each individual patient but with the recent more intensive treatment of hypertension, the time from the onset of clinical albuminuria to death has virtually trebled from 7 to 21 years (Astrup et al., 2005) despite a considerable number of patients (45%) still reaching ESRD or dying before reaching ESRD (10%) (Forsblom et al., 2011; Rosolowsky et al., 2011).

Extrarenal complications and their relationship with renal involvement

Cardiovascular disease and the cardiorenal syndrome

Albuminuria independently associates with increased cardiovascular mortality and morbidity in patients with diabetes (Borch-Johnsen et al., 1987; Deckert et al., 1992; Ruggenenti et al., 2012d), hypertension (Bianchi et al., 1999; Ibsen et al., 2005), and in the general population (Vestbo et al., 1995; Gerstein et al., 2001; Hillege et al., 2002; Arnlov et al., 2005) and therefore reflects not only a sign of renal impairment and a key pathogenic element of renal disease progression but also a marker of more generalized vascular damage.

Many studies have linked albuminuria with cardiovascular disease. In particular, in the Microalbuminuria, Cardiovascular and Renal Outcomes of the Heart Outcomes Prevention Evaluation (MICRO-HOPE) study, patients with microalbuminuria at baseline had an increased risk of cardiovascular disease compared with normoalbuminuric ones, irrespective of their diabetic status (Gerstein et al., 2001), and a linear relationship was observed between albumin excretion and cardiovascular disease risk even within the normal range (Ruggenenti and Remuzzi, 2006). Patients with type 1 diabetes and albuminuria have about 10 times higher relative mortality from cardiovascular disease when compared to similar patients with normoalbuminuria (Borch-Johnsen and Kreiner, 1987; Tuomilehto et al., 1998), who in turn have approximately two times higher similar risk for cardiovascular death than the general non-diabetic population (Rossing et al., 1996b). Patients with type 2 diabetes with microalbuminuria or macroalbuminuria retain respectively a 1.7 and 2.6 times higher risk for cardiovascular mortality when compared with normoalbuminuric patients (Gerstein et al., 2001; Ibsen et al., 2006).

The exact significance and mechanism of the relationship between albuminuria and cardiovascular disease are not yet understood. However, once developed, microalbuminuria not only denotes renal capillary damage but also represents an early biological marker of vascular injury (Deckert et al., 1989). Endothelial dysfunction (Karalliedde and Gnudi, 2011) has been implicated as a potential major mechanism for vascular disease and microalbuminuria has been linked to markers of endothelial dysfunction in patients with and without diabetes (Stehouwer and Smulders, 2006). Findings that, in patients with diabetes and mild renal insufficiency, microalbuminuria has been associated with abnormal vascular remodelling, independent of blood pressure (Hermans et al., 2008), and higher levels of inflammatory molecules such as C-reactive protein and fibrinogen (Parving et al., 2001), can be taken to suggest the possibility of a link between markers of vascular disease and microalbuminuria (Persson et al., 2006, 2008; Menne et al., 2010).

UAER is related in an exponential fashion with cardiovascular risk with no evidence for a threshold in both the general healthy population and patients with diabetes (Gerstein et al., 2001; Karalliedde and Viberti, 2004; Ruggenenti et al., 2012d), and increasing levels of urinary albumin associated with incremental risk of cardiovascular events even in the normoalbuminuric range (Ruggenenti et al., 2012d). In subjects with type 2 diabetes and overt nephropathy, macroalbuminuria show a greater incidence of stroke and CHD, worse left ventricular systolic function, impaired diastolic left ventricular filling, and a lower survival rate than subjects with lower UAER (Miettinen et al., 1996; Liu et al., 2003). On the other hand, treatment with ACEIs or ARBs is associated with a reduction in proteinuria and lower cardiovascular risk in patients with type 1 or type 2 diabetes and micro- or macroalbuminuria (Hovind et al., 2001; de Zeeuw et al., 2004; Ruggenenti et al., 2011a) and in non-diabetic individuals with micro- or even normoalbuminuria (Schmieder et al., 2011).

Retinopathy

Diabetic retinopathy is an important manifestation of chronic microvascular disease in diabetes and is currently managed when the retinal lesions are found in advanced stages with topical treatments such as laser photocoagulation. Diabetic retinopathy is the major cause of blindness in the Western world and results from the Diabetes Control Complications Trial (DCCT), United Kingdom Prospective Diabetes Study (UKPDS), and STENO study have demonstrated that metabolic and blood pressure control play an important role in its progression (Writing Team for the DCCT, 2002; Gaede et al., 2008; Kohner, 2008). In diabetes, CKD is a risk factor for prevalent retinopathy and clinical proteinuria is a recognized risk factor for incident retinopathy (Agarwal et al., 2012), which suggests a similar pathogenic mechanism of renal and eye disease in diabetes. Indeed growth factors such as VEGF-A, implicated in the pathophysiology of retinopathy, have been found to be regulated by angiotensin II (Zhang et al., 2004), and it has been postulated that the RAAS could play a key role in the diabetic retina, and that retinal protection may be afforded by therapeutic blockade of the RAAS.

The EUCLID (EUCLID Study Group, 1997) and BENEDICT (Ruggenenti et al., 2004) trials showed that ACEI therapy may have a beneficial effect on onset and progression of retinopathy in type 1 and type 2 diabetic patients, respectively. The DIRECT study subsequently showed that ARB therapy may also be beneficial in this population (Chaturvedi et al., 2008; Sjolie et al., 2008).

Similarly, in the RASS study, the blocking of the RAAS in type 1 diabetic subjects demonstrated a reduction in the development and progression of diabetic retinopathy with inhibition of RAAS with both the ACEI enalapril and the angiotensin II receptor antagonist losartan (Mauer et al., 2009). In hypertensive, normoalbuminuric type 2 diabetes patients included in the BENEDICT trial, the ACEI

trandolapril achieved regression of retinopathy in a substantial proportion of those with established retinal involvement at inclusion, a finding that combined to the protective effect of treatment against new onset of microalbuminuria, appeared to reflect a generalized beneficial effect of RAAS inhibition on the diabetic microvasculature (Ruggenenti et al., 2010a). This finding is in apparent contrast with data from the EUCLID, DIRECT, and RASS trials, that failed to show a similar association between retinal benefits and renoprotection. However, the above trials included patients with normal blood pressure at very low risk of transition from normo- to microalbuminuria over the observation period. Thus, they were likely underpowered to demonstrate any renoprotective effect in this context (Bilous et al., 2009).

Correction of dyslipidaemia by statins, especially if combined with fenofibrate, has also been shown to ameliorate retinopathy; further topic treatment with anti-VEGF-A agents has become a novel treatment option in advanced retinopathy (Abbate et al., 2011), but caution is recommended for potential risk of systemic and renal adverse events (Pelle et al., 2011).

In conclusion, patients with diabetes, CKD, and proteinuria should undergo regular retinal examinations in order to protect sight.

Neuropathy

Patients with diabetes and CKD stages 4 and 5 often present with neurologic complications such as peripheral neuropathy and cardiovascular autonomic neuropathy (Zander et al., 1989; Fernando et al., 1991). In these patients, renal involvement is often severe because of the long duration of diabetes and, even most important, the synergistic effect on peripheral nerves of uraemic toxins in addition to those of hyperglycaemia.

Diabetic peripheral neuropathy is a common chronic complication and is associated with high morbidity, high risk of lower-extremity amputation, and poor quality of life (Tesfaye et al., 2010). Similarly, cardiovascular autonomic neuropathy is associated with life-threatening consequences, such as silent myocardial ischaemia and increased mortality (Navarro et al., 1991; Rathmann et al., 1993), a phenomenon much aggravated by progressive later stages of CKD (Pop-Busui et al., 2010) and representing the single greatest cause of mortality in patients with ESRD on dialysis (Ranpuria et al., 2008).

Uraemic neuropathy presents as a distal symmetrical polyneuropathy with greater lower-limb than upper-limb involvement—the longer the nerve, the more severe is the disease—and manifests with severe burning and shooting sensations with altered pain and temperature perception, paraesthesia, impaired or absent deep reflexes, impaired vibration sense, often paralleled by muscle wasting, and weakness (Krishnan and Kiernan, 2007). Motor nerve dysfunction is not often seen, and in some cases neuropathy can be paralleled by ulceration or neuroarthropathy (Charcot joints) of the foot, complications more prevalent in patients with diabetes and ESRD (Foster et al., 1995).

Cardiac autonomic neuropathy is often characterized by abnormal heart rate variability, tachycardia at rest, exercise intolerance, and orthostatic hypotension, which often manifest with exercise intolerance mainly secondary to blunted exercise-mediated increases in cardiac output (Giordano et al., 2001; Colberg et al., 2003). Orthostatic hypotension (The Consensus Committee of the American Autonomic Society and the American Academy of Neurology, 1996) is seen in diabetic patients mainly because of efferent sympathetic vasomotor denervation, which, by determining a reduced vasoconstriction of the splanchnic and other peripheral vascular beds, results in light-headedness, weakness, dizziness, and, in the extreme cases, syncope on standing.

Cardiac autonomic neuropathy also has important prognostic implications mainly for silent myocardial ischaemia. Patients lack or present with decreased symptoms for ischaemic pain, which results in delayed or missed recognition of myocardial ischaemia/infarction and appropriate therapy (Vinik and Ziegler, 2007). These patients often show abnormal myocardial perfusion and blood flow regulation (Di Carli et al., 1999) which often can precipitate in malignant arrhythmogenesis and cardiac death (Lown and Verrier, 1976; Willich et al., 1993).

The mechanisms inducing neurotoxicity (peripheral neuropathy and cardiac autonomic neuropathy) in patients with diabetes and CKD is multifactorial and still not fully dissected. The complex interactions between diabetes-related metabolic and haemodynamic perturbation have been implicated together with disease duration and age (Stella et al., 2000; Witte et al., 2005), and, specifically in patients with CKD, metabolic perturbations are paralleled by the superimposed uraemic state with its constellation of unique metabolic/physiologic alterations which contribute to an accelerated onset and progression of nerve damage. Both parathyroid hormone and β_2-microglobulin (increased in patients with ESRD) have been implicated in the development of uraemic neuropathy (Massry, 1987; Vanholder et al., 1994), and chronic hyperkalaemia, as seen in these patients, has also been proposed as an important player in the pathogenesis of neuropathy, promoting nerve depolarization (Krishnan et al., 2005).

Until recently, the only effective proven therapeutic approach for nervous system dysfunction is prevention with strict glycaemic control as shown by the DCCT (The DCCT Research Group, 1993, 1998) and the EURODIAB (Tesfaye et al., 1996) in patients with type 1 diabetes, although these results do not seem to be translatable to type 2 diabetes (Callaghan et al., 2012). Of interest, in a recent study in hypertensive patients with type 2 diabetes, the ACEI delapril alone or combined with the third-generation calcium channel blocker (CCB) manidipine associated with a reduced incidence of new-onset neuropathy in those without neural involvement or an accelerated regression in those with established disease at inclusion (Ruggenenti et al., 2011b). The finding that improved outcome was associated with amelioration of the glucose disposal rate compared to placebo was taken to suggest that treatment effect could be at least in part explained by improved insulin resistance. The possibility of an improved microcirculation and neural perfusion associated with RAAS inhibition is also a plausible explanation. Whether this clinical benefit in early stages of the disease may translate into effective prevention of hard endpoints, such as the need for amputation and fatal and non-fatal cardiac arrhythmias in patients with more severe peripheral neuropathy, merits further scrutiny.

It is unclear whether metabolic and haemodynamic treatment for neuropathy is still a good strategy in patients with advanced CKD or on dialysis who, because of all co-morbidities, remain a group at elevated morbidity and mortality.

Anaemia

Chronic anaemia is commonly found in patients with diabetes and CKD (Thomas et al., 2004a, 2004b); patients with diabetes

appear to be more prone to develop anaemia (two- to threefold) than patients with different CKD aetiology and, at comparable levels of residual renal function, anaemia is invariably more severe in patients with diabetes than in those without (Astor et al., 2002). Anaemia is found earlier in patients with diabetes than in those with CKD from other causes (Thomas et al., 2003) and it may occur in the absence of significant renal impairment (Craig et al., 2005; Al-Khoury et al., 2006). Microalbuminuria and increasing levels of albuminuria are associated with progressively higher prevalence of anaemia which seems not to be related to impairment in renal function (Adetunji et al., 2009).

The relationship between diabetes and anaemia has been related to advancing kidney damage involving the tubulointerstitial compartment and deficiency of and/or resistance to erythropoietin (Macdougall and Cooper, 2002; Thomas et al., 2003; Al-Khoury et al., 2006). The failure to increase circulating erythropoietin levels in response to falling haemoglobin levels is the dominant factor in the genesis of anaemia associated with DN as it is with CKD (Inomata et al., 1997; Winkler et al., 1999), a phenomenon that appears of greater magnitude in diabetes rather than that seen in other renal diseases affecting predominantly the glomeruli (Astor et al., 2002). Inflammation, reduced red cell survival, and autonomic neuropathy have also been implicated with links to anaemia in diabetes (Thomas et al., 2005).

Chronic anaemia identifies patients with diabetes at increased risk for adverse outcomes such as higher cardiovascular morbidity and mortality, and hospitalization, independent of the presence or severity of nephropathy (Keane and Lyle, 2003; Rossing et al., 2004).

The Anaemia Correction in Diabetes (ACORD) (Ritz et al., 2007) trial in patients with diabetes and CKD, which targeted haemoglobin levels of 13–15 mg/dL, found no favourable or adverse effects on cardiovascular mortality or morbidity over a 2-year follow-up. Even more importantly, the Trial to Reduce cardiovascular Events with Aranesp Therapy (TREAT), aimed to achieve near-normal haemoglobin levels in patients with type 2 diabetes and CKD, failed to show any benefit on either of the two primary composite outcomes—composite outcomes of death or a cardiovascular event, and of death or ESRD—with darbepoetin compared to placebo. Moreover, darbepoetin treatment nearly doubled the risk of stroke compared to placebo (Marsden, 2009; Pfeffer et al., 2009). Even if anaemia is causally involved, the pathogenesis of cardiovascular disease in patients with diabetes and CKD is so complex that addressing just one factor (anaemia) may not suffice to prevent cardiovascular risk, and fully normalizing haemoglobin levels may even be harmful (Abaterusso et al., 2008).

Bone disease

In CKD, patients with diabetes appear to be more prone to bone-related abnormalities than non-diabetic ones; patients with type 1 diabetes present with more significant decreases in bone mineral content when compared with age- and sex-matched non-diabetic subjects. Data in type 2 diabetes are less consistent.

The reduction in bone mineral content seen in patients with type 1 diabetes is often associated with longer disease duration, is more pronounced in patients with pre-pubertal or pubertal onset, and with poor glycaemic control. These alterations of bone metabolism have been described as adynamic bone disease (McNair, 1988), a condition characterized by significant reduction in bone turnover, associated with lower parathyroid hormone levels (a so-called state of functional hypoparathyroidism) leading to osteopenia, increased fracture risk, and cardiovascular calcification, secondary to the inability of calcium and phosphorus in forming bone tissue, becoming thus available for deposition in ectopic sites such as vessels, worsening coronary and peripheral artery disease (Braun et al., 1996; London et al., 2003, 2004) and increased mortality (Avram et al., 2001; Guh et al., 2002). Importantly low intact parathyroid hormone (iPTH) levels have been prospectively related to increased mortality in uraemic patients, independently of diabetic status or duration of diabetes (Avram et al., 2001; Stevens et al., 2004). The clear pathophysiology of this condition in patients with diabetes and CKD is still unclear, but, especially in diabetics, it may present earlier than non-diabetic patients, before advance CKD stages.

Among patients on haemodialysis, those with diabetes show lower iPTH levels compared to non-diabetics (Inaba et al., 2001, 2002), and, within diabetics, an inverse correlation between iPTH serum levels and glycaemic control has been described. Specifically, diabetic patients with HbA1c levels ranging from 7% to 8% have low circulating iPTH levels, while higher levels are conversely found in diabetic patients with HbA1c levels of 5–6% (Murakami et al., 2008).

In this context, impaired iPTH secretion appears to be the main determinant responsible for decrease bone turnover, as similar degree of iPTH biological action on bone in the diabetic and non-diabetic population has been described (Inaba et al., 2002). Studies have suggested that poor metabolic control per se, via increases in advanced glycation end-products, could inhibit low calcium-mediated iPTH secretion (Yamamoto and Ozono, 2001). Of interest, leptin, a marker of adipose mass found to be raised in diabetic obese patients (Fruhbeck et al., 1998), is linked to bone mass, and has been proposed to reduce bone turnover in dialysis patients (Zoccali et al., 2004). Indeed leptin induces vascular calcifications *in vitro* and has been associated with cardiovascular events in overweight and obese dialysis patients (Mallamaci et al., 2005). Pro-inflammatory cytokines, increased in insulin-resistant conditions such as diabetes and in uraemic conditions, have been proposed as potential players in the pathogenesis of both vascular (Stenvinkel et al., 2002) and bone disease (Gal-Moscovici et al., 2002; Moffett et al., 2004).

Bone and cardiovascular disease is clearly linked and should not be forgotten in all renal patients approaching or on haemodialysis, especially in patients with diabetes.

Risk factors for disease onset and progression

Inborn risk factors

Evidence of familial clustering strongly suggests that genetic factors are important in determining susceptibility for DN (Seaquist et al., 1989; Borch-Johnsen et al., 1992). In cross-sectional studies, diabetic siblings of probands with type 1 diabetes and DN have a prevalence of DN of between 33% and 83%, as compared to a prevalence of 10– 19% in siblings of probands without DN (Seaquist et al., 1989; Borch-Johnsen et al., 1992; Quinn et al., 1996). Thus a 4.5–8-fold risk of developing DN has been quoted for diabetic siblings of probands with DN. The reported cumulative incidence

of DN in siblings with type 1 diabetes of a proband with type 1 diabetes and DN is 71.5%, as compared with 25.4 % in siblings of a proband unaffected by DN (Quinn et al. 1996). It is thought that DN is a complex trait in which a genetic predisposition may interact with environmental determinants but the nature of the genetic susceptibility remains unknown. Several genes have been proposed as biologically plausible candidates for the 'nephropathy gene' based upon the known pathophysiology of DN but to date no 'clear-cut' genetic signature has been identified (Freedman et al., 2007).

There is significant evidence of the importance of systemic blood pressure elevation in DN and of an excess of hypertension and cardiovascular disease in relatives of diabetic patients with DN compared with those without (Krolewski et al., 1988; Earle et al., 1992). The suggestion that DN may form part of a wider propensity to cardiovascular disease (Barzilay et al., 1992) has made investigators focus their attention on genes involved in blood pressure control and cardiovascular disease, in particular those involved in the RAAS.

Analyses of the role of polymorphisms within the *ACE* gene (Wang et al., 2012), and other genes implicated in the pathophysiology of DN have been inconclusive. Recent work in type 2 diabetic patients has identified regions on chromosomes 2, 3, 7, 13, 16, and 22 suggestive for linkage with DN (Freedman et al., 2008; Igo et al., 2011). This work will potentially lead to the identification of multiple genes involved in susceptibility for nephropathy in subjects with diabetes. Many of these observations however remain unconfirmed or still controversial (Freedman et al., 2007). The emerging picture seems to suggest a complex interaction between the effects of several genes and multiple environmental factors.

Treatable risk factors

Hyperglycaemia

Type 1 diabetes

Poor glycaemic control has been recognized as one of the major determinants of progressive kidney disease in diabetes. Prospective randomized controlled trials such as the DCCT in patients with type 1 diabetes demonstrated that improved glycaemic control resulted in a 39% and 54% reduction in microalbuminuria and macroalbuminuria respectively (The DCCT Research Group, 1993). The relationship between glycaemia and the risk of microalbuminuria did not show evidence for a threshold below which improved glycaemic control could not further reduce the risk of kidney disease (Genuth, 2012).

At the conclusion of the DCCT, participants were informed about the results, and were encouraged to follow the intensive treatment. These patients were followed in the Epidemiology of Diabetes Interventions and Complications (EDIC) study for a further 7 years (median follow-up period of 22 years in the combined studies); results showed that the previous intensive treatment of diabetes with near-normal glycaemia during the DCCT had an extended benefit on the progression of nephropathy and that early sustained intensive glycaemic treatment in patients with type 1 diabetes provides long-term beneficial effects on the risk of nephropathy (Writing Team for the DCCT, 2003; de Boer et al., 2011a, 2011b). Importantly in this study (de Boer et al., 2011a, 2011b) the effect of intensive glycaemic control on the risk of renal disease progression was almost completely abrogated after statistical adjustment for albuminuria, making albuminuria fit one of the primary criterion for a surrogate endpoint of renal disease in diabetes (Perkins, 2012).

Type 2 diabetes

In newly diagnosed patients with type 2 diabetes, the UKPDS demonstrated that intensive glycaemic control reduced the risk for the development of albuminuria or clinical albuminuria by about 33%, and also significantly reduced the proportion of patients doubling their plasma creatinine over 12 years (The DCCT Research Group, 1995). Similarly to the EDIC study, a continued reduction in microvascular risk was observed during 10 years of UKPDS post-trial follow-up (Holman et al., 2008a).

The ACCORD (Gerstein et al., 2008), ADVANCE (Patel et al., 2008), and the VADT (Duckworth et al., 2009) trials, were three clinical trials enrolling respectively 10,251, 11,140, and 1791 type 2 diabetics with approximately 10 years' duration of disease, poor glycaemic control (HbA1c ~ 9%), and high vascular risk, which evaluated whether an intensive glycaemic control (HBA1c < 6–6.5%) had any significant effects on micro- and macrovascular disease outcomes after a follow-up variable between 3.4 and 6 years (Dluhy et al., 2008; Agrawal et al., 2011). Only the ADVANCE trial found a positive effect of intensified metabolic control on nephropathy (Poulter, 2009). Overall, data failed to demonstrate any appreciable benefit of intensified treatment on both micro- and macrovascular complications.

The above findings, combined with evidence of an excess risk of hypoglycaemia episodes in patients on intensified therapy, challenge the need of reducing HBA1c below the currently recommended targets of 7% in most diabetic patients. Physicians might reasonably suggest more stringent HBA1c goals, such as 6.5%, for selected individual patients, if this can be achieved without significant hypoglycaemia or other adverse effects of treatment (American Diabetes Association, 2012b).

Hypertension

High blood pressure is considered a real treatment target for patient with diabetes as it not only translates into a significant insult to the diabetic glomeruli but might also worsen and amplify the metabolic insults to the glomerulus (Gnudi et al., 2007). Moreover, hyperglycaemia-induced pre-glomerular vasodilation may favour the transmission of systemic hypertension to the glomerular microvasculature with glomerular hypertension and consequent damage. This may explain the major role of raised arterial blood pressure in the development and progression of diabetic kidney disease. High blood pressure levels predict, in patients with type 1 diabetes, the development of microalbuminuria, and paternal hypertension predicts the risk of DN in the offspring (Fagerudd et al., 1998). Similarly, in Pima Indians with type 2 diabetes, elevated blood pressure before the occurrence of diabetes predicts albuminuria after the onset of the disease (Nelson et al., 1993). In prospective studies, patients with diabetes who progress to albuminuria have higher arterial pressure at baseline (Haneda et al., 1992; The Microalbuminuria Collaborative Study Group, United Kingdom, 1999), and in intervention studies blood pressure lowering slows kidney disease progression and reduces albuminuria in both type 1 and type 2 diabetes (Lewis et al., 1999; Pohl et al., 2005).

In the UKPDS, the effect of tighter blood pressure control in patients with type 2 diabetes and hypertension reduced the

occurrence of microalbuminuria, but, in contrast to glycaemic control, no legacy effect has been observed for blood pressure, and therefore good blood pressure control must be continued if the benefits are to be maintained (Holman et al., 2008b).

Importantly the benefit of intensive treatment with tighter metabolic and haemodynamic targets has been demonstrated in the STENO-2 study. In this study, patients with type 2 diabetes were followed for a median of 7.8 years: the decline in HbA1c, systolic and diastolic blood pressures, and lipid levels were uniformly larger in the intensive-therapy than in the conventional-therapy group (Gaede et al., 2003). Intensive therapy significantly lowered the risk of micro- and macrovascular complications by nearly 50%. Antihypertensive therapy and improved glycaemic control were independent predictors for reduction of UAER which was paralleled by preservation of kidney function (Gaede et al., 2004).

In the above study, however, < 20% of patients in the intensive arm achieved the pre-established HbA1c targets (HbA1c < 6.5%), whereas 60–70% of patients achieved the blood pressure and lipid targets. The finding that benefits on cardiovascular disease outcomes remained significant in both the short- (7.8 years) and the subsequent long-term follow up assessment at 13 years, even when mean HbA1c levels assessed at the later time point had merged and were similar at around 8% for both treatment arms, can be taken to suggest that blood pressure and lipid control might impact more than intensified metabolic control on chronic complications of diabetes, a hypothesis that is consistent with the negative findings of ACCORD, ADVANCE, and VADT (Gaede et al., 2003, 2008).

Smoking

Smoking is a risk factor for anticipated onset of microalbuminuria in patients with diabetes (Sawicki et al., 1994; Cignarelli et al., 2008). In type 2 diabetes, smoking correlates with degree of renal impairment, and the progression of diabetic kidney disease is up to twofold faster in smokers than in non-smokers (Biesenbach et al., 1997; Cignarelli et al., 2008). Smoking cessation is in turn associated with reduction of microalbuminuria in both type 1 and 2 diabetes (Sawicki et al., 1994; Voulgari et al., 2011).

Obesity

Excess weight, and in particular visceral obesity, is a well-recognized phenotypic characteristic and driver for type 2 diabetes, and at the same time a risk factor for DN (Hall et al., 2003). Visceral obesity is a major component of the metabolic syndrome, which is characterized by insulin resistance, hypertension, dyslipidaemia, and increased renal and cardiovascular morbidity. Insulin resistance precedes and likely contributes to the onset of microalbuminuria in patients with type 1 diabetes (Orchard et al., 2002). In type 2 diabetes, there is a clear association between more severe insulin resistance and microalbuminuria, that is also evident in subjects with normal blood pressure values considered separately from those with hypertension (Parvanova et al., 2006). Visceral obesity per se is associated with activation of the RAAS and physical compression of the kidneys (Henegar et al., 2001; Hall et al., 2004), which in turn sustains an increase in systemic and glomerular pressure leading to renal injury in a similar manner as seen in diabetes. Obesity in humans (Chagnac et al., 2003) is paralleled by hyperfiltration, secondary to increased tubular salt reabsorption leading to tubuloglomerular feedback-mediated reduction in afferent arteriolar resistance, and increased glomerular capillary pressure (Hall, 2003), a phenomenon likely to add to the negative effect of diabetes on glomerular haemodynamics (Griffin et al., 2008). Analyses of dextran sieving data underscore that the glomerular capillary bed of obese patients is exposed to increased perfusion and higher transcapillary hydraulic pressure (Chagnac et al., 2003). In turn, glomerular hypertension and hyperfiltration may enhance urinary albumin excretion. It is likely that the obesity and diabetes-mediated states of low-grade inflammation, associated with kidney macrophage infiltration and increased oxidative stress, are factors that together contribute additively to the progression of renal disease in obese patients with diabetes.

Dyslipidaemia

Hyperlipidaemia is known to be a risk factor for the development of albuminuria in patients with diabetes (Rutledge et al., 2010). However, conflicting evidence exists on the role of lipid-lowering agents, particularly statins, in improving albuminuria and preserving renal function in patients with DN (Fried et al., 2001; Ruggenenti et al., 2010b; Rutter et al., 2011). Post hoc analysis of studies on the role of fibrates on DN in type 2 diabetes showed a positive effect on renal function decline but not albuminuria (Forsblom et al., 2010). Further prospective studies are needed to assess the role of hypolipaemic treatment of the progression of DN.

Diagnosis and monitoring

Measurement of albuminuria/proteinuria

The clinical diagnosis of diabetic renal disease is based upon the detection of albuminuria (Fig. 149.7) Determination of albuminuria, blood pressure assessment, and estimation of GFR are the parameters that are utilized as diagnostic procedures for DN. In selected cases, mainly because of unusual presentation of renal disease, renal biopsy may serve to exclude other underlying renal disease conditions.

In patients with type 2 diabetes, because of the difficulty in precisely dating the onset of diabetes, screening for DN must be performed at the time of diagnosis, when it has been reported that approximately 7% of patients will already have microalbuminuria (Adler et al., 2003).

In patients with type 1 diabetes, microalbuminuria rarely occurs close to diagnosis or after a short diabetes duration; therefore, screening is advised after 5 years from diagnosis (Molitch et al., 2004). Of note, it is important to remember that, especially in patients with poor metabolic and haemodynamic control, the prevalence of microalbuminuria before 5 years can reach 18% (Stephenson et al., 1994). Some evidence suggests that the pre-pubertal duration of diabetes may contribute to the development of microvascular complications and this should be considered in individualizing recommendations in different patients (Schultz et al., 1999).

After the initial screening and in the absence of previously demonstrated microalbuminuria, a test for the presence of microalbuminuria should be performed annually (Molitch et al., 2004).

Screening for microalbuminuria can be performed by albumin in a spot urine sample, collected either as the first urine in the morning or at random. This method is accurate and easy to perform, in contrast to 24-hour and timed urine collections which are prone

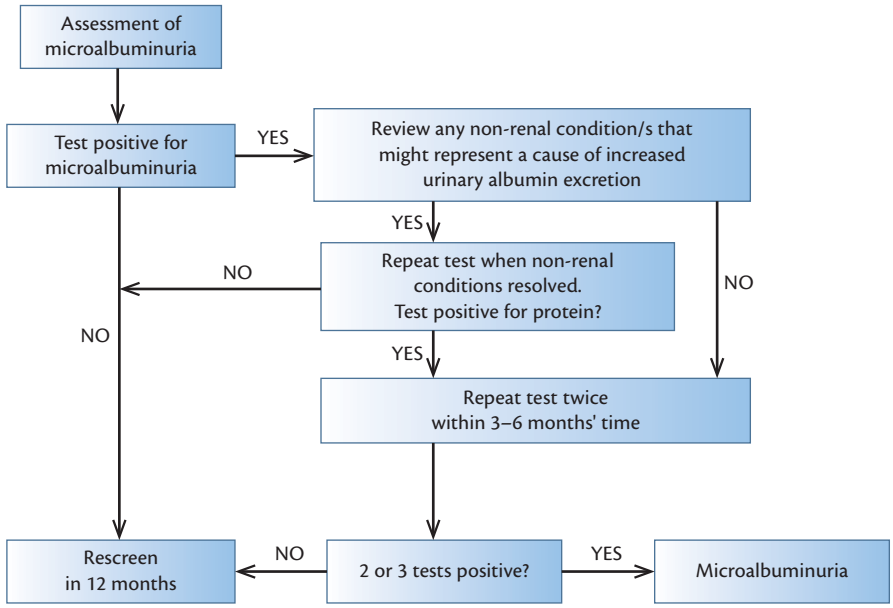

Fig. 149.7 Screening for microalbuminuria.
Modified from Molitch, M. E., DeFronzo, R. A., Franz, M. J., *et al.* (2004). Nephropathy in diabetes. *Diabetes Care*, 27 Suppl 1, S79–83.

to errors related to collecting samples and cumbersome (Molitch et al., 2004). The result of albumin measurements in spot collections is better expressed as urinary ACR (mg/g or mg/mmol) (Eknoyan et al., 2003; Molitch et al., 2004). All abnormal tests must be confirmed in two out of three samples collected over a 3–6-month period due to the known day-to-day variability in urinary albumin excretion (Eknoyan et al., 2003; Molitch et al., 2004). Screening for urine albumin should not be performed when conditions known to transiently increase urinary albumin excretion are present, such as urinary tract infections, hyperglycaemia, haematuria, acute febrile illness or prolonged and strenuous exercise, uncontrolled hypertension, and heart failure (Mogensen et al., 1995). Samples should always be refrigerated if analysed at a later stage (Eknoyan et al., 2003). Of the many ways of determining albuminuria in everyday clinical practice, measurement of the ACR in a first-morning void is believed to be superior in predicting renal events in patients with type 2 diabetes and nephropathy (Lambers Heerspink et al., 2010). Measurements in accurate overnight or 24-hour urine collections remain the gold standard to assess albuminuria as a quantitative outcome or endpoint intervention in the context of clinical trials.

When specific urinary albumin excretion determinations are not available, urine protein dipsticks can be utilized, but all negative tests should be confirmed by more specific methods as this method is not sensitive enough to detect small increments in urinary albumin (Molitch et al., 2004).

Although albumin determination in the urine is the fundamental step utilized for the diagnosis of DN, patients with either type 1 or type 2 diabetes can present with reduced renal function but still normal urinary albumin excretion (Caramori et al., 2003; MacIsaac et al., 2004). In up to 30% of type 2 diabetic patients, renal impairment may not be associated with concomitant micro- or macroalbuminuria and retinopathy (Kramer et al., 2003). Ageing-related changes in kidney structure and function and/or predominantly ischaemic kidney disease might possibly explain why renal function may be impaired in patients with no evidence of microvascular disease (microalbuminuria or retinopathy). Independent of the above, in addition to the assessment of albuminuria, a careful GFR measurement is therefore needed whenever diabetic patients are screened for renal disease.

Determination of glomerular filtration rate

The GFR can be directly evaluated by measuring the renal or plasma clearance of exogenous markers of glomerular filtration such as inulin, ^{51}Cr-EDTA, ^{125}I-iothalamate, and iohexol (Gaspari et al., 1997), or by measuring the creatinine clearance, an endogenous marker of glomerular filtration that, however, is either secreted or absorbed at tubular level, which affects the reliability of creatinine-based GFR measurement (Friedman et al., 1988).

In clinical practice, GFR is often estimated by equations that take into account serum creatinine concentration and some or all of the following variables: age, gender, race, and weight. The currently recommended equation, utilized worldwide, is the MDRD (Modified Diet in Renal Disease) equation (Levey et al., 2006), calculated as follows:

$$\text{GFR (mL/min/1.73 m}^2) = 186 \times (\text{serum creatinine (mg/dL)})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American}).$$

This equation is more precise in a GFR range < 60 mL/min and is extensively utilized to monitor the progression towards ESRD in patients with renal impairment. On the other hand, estimation formulas have been proven to be grossly inaccurate and unreliable in different clinical settings, including polycystic kidney disease and diabetes, particularly in the presence of normal or increased estimated GFR (eGFR) values (Ruggenenti et al., 2012c, 2012e). In 2009, the Chronic Kidney Disease Epidemiology (CKD-EPI) Collaboration equation was developed to specifically address this limitation, but the diagnostic performance of this novel equation in diabetic patients is also questionable (Rognant et al., 2011; Silveiro et al., 2011; Ruggenenti et al., 2012c, 2012e). Moreover, recent data

question the use of any estimation formula to identify hyperfiltering subjects and monitor renal function loss in patient with type 2 diabetes with normo- or microalbuminuria (Gaspari et al., 2013). Since hyperfiltering diabetics are at increased risk of accelerated renal function loss and progression to micro- or macroalbuminuria and since GFR decline over time is remarkably faster in diabetics than in non-diabetics even before the onset of overt nephropathy (a loss largely underestimated or fully missed by estimation formulas), appropriate measurement of true GFR could be advisable to monitor both disease progression and response to treatment in diabetic patients. In particular, in patients with renal insufficiency, increased tubular creatinine secretion results in GFR overestimation. In those with oedema, haemodilution may result in reduced serum creatinine levels, which may also result in GFR overestimation. On the other hand, accurate measurement of GFR and detection of hyperfiltration have a significant prognostic relevance in diabetic patients (Ruggenenti et al., 2012c).

Treatment and management of the patient with diabetes and renal disease

Along with intensive glycaemic control (The DCCT Research Group, 1993; UK Prospective Diabetes Study Group, 1998; EDIC Study Group, 2000), antihypertensive treatment is a cornerstone of the prevention and treatment of DN (Table 149.3). In particular, RAAS blocking agents have been extensively used based on strong evidence of their efficacy in experimental diabetes. Although normo-, micro-, and macroalbuminuria are part of a continuum in the relationship between albumin excretion and cardiorenal risk (Gerstein et al., 2001; Ruggenenti and Remuzzi, 2006), trials of blood glucose or blood pressure-lowering agents and RAAS inhibitors have almost invariably considered patients selected and categorized on the basis of a UAER in a pre-defined range, that is in the range of normo-, micro-, or macroalbuminuria. This approach was aimed at including patients with a homogenous and predictable risk of events and, at the same time, allowed trial endpoints to be better defined. Thus, largest trials considered as a primary endpoint categorical variables such as progression to micro- or macro-albuminuria (taken as markers of incipient or overt nephropathy, respectively (Parving, 2000; Reboldi et al., 2011)) or to ESRD for patients with normo-, micro-, or macro-albuminuria, respectively. Regression from micro- to normoalbuminuria was also a secondary outcome of some trials. Smaller studies considered albuminuria as a continuous variable and reduction of albuminuria was the main outcome of these studies. The rate of GFR decline was seldom considered as the primary outcome, most likely because of the difficulties in serially measuring GFR by appropriate techniques in a sufficient number of patients. Mortality and cardiovascular events were also considered as outcomes in largest trials, in particular in high-risk patients with overt nephropathy. To describe the enormous number of trials evaluating the effect of different pharmacological interventions in patients with diabetes, we categorized the studies according to the typology of considered patients and, secondarily, according to intervention targets and considered outcomes.

Preventing microalbuminuria in patients with normoalbuminuria

Metabolic control

Type 1 diabetes

Metabolic control plays a key role in preventing microalbuminuria in both type 1 and type 2 diabetes. The DCCT, a landmark trial conducted from 1983 to 1993, aimed to evaluate whether intensive

Table 149.3 Blood pressure targets and antihypertensive drugs in different populations of patients with or without diabetes

Patients	BP target (mmHg)	Recommended drugs	Guidelines	Comments
No evidence of renal disease No diabetes Diabetes	<140/90 <130/80	β-blockers, CCBs, Diuretics ACEIs[a] ACEIs ARBs	KDOQI, 2004 ADA, 2003 KDOQI, 2004	No additional renoprotection with lower BP levels Choice of antihypertensive agents according to patient's characteristics ACEIs prevent or delay incidence of microalbuminuria Crucial role of optimized metabolic control
CKD without proteinuria No diabetes Diabetes	<130/80 <130/80	ACEIs[a] ACEIs[a] ARBs[a]	KDOQI, 2004 KDOQI, 2004	Reduction of BP prevent progression of renal injury but no lower than 130/80 mmHg Addition of aldosterone antagonist or rennin inhibitor can be considered to maximize renoprotection
CKD with proteinuria No diabetes Diabetes	<130/80 <125/75	ACEIs[a] ACEIs ARBs ACEIs + ARB	KDOQI, 2004 ADA, 2003	ACEIs prevent or delay incidence of microalbuminuria Crucial role of optimized metabolic control—BP reduction with maximal doses of ACEIs and ARBs reduces proteinuria and the rate of renal disease progression.

[a]Consider salt restriction and addition of diuretics to the suggested treatment.

ADA = American Diabetes Association; ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; BP = blood pressure; CCB = calcium channel blocker; KDOQI = Kidney Disease Outcomes Quality Initiative.

From Cravedi, P., Ruggenenti, P., and Remuzzi, G. (2010). Which antihypertensive drugs are the most nephroprotective and why? *Expert Opin Pharmacother*, 11, 2651–63.

metabolic control may delay or even prevent diabetes-related microvascular complications, including DN. The primary intervention cohort included 726 subjects with type 1 diabetes and normoalbuminuria, who were randomized to either intensive glycaemic control (target HBA1c: 6% or less) or standard glycaemic control. Intensive control was associated with a significant 34% reduction in the risk of new-onset microalbuminuria compared to standard glycaemic control over an average of 6.5 years of follow-up (The DCCT Research Group, 1995). These findings were extended by the EDIC study, an observational, long-term follow-up of the surviving members of the original DCCT cohort (The EDIC study group, 2003). At the end of the DCCT, all patients were shifted to intensive metabolic control, and their care was transferred to their own physicians. Of note, analyses of the 1112 subjects with normal AER at both the beginning and the end of DCCT showed that only 39 out of 572 participants (6.8%) originally assigned to intensive metabolic control developed microalbuminuria at the EDIC years 7 or 8 compared to 87 out of 550 subjects (15.8%) originally assigned to standard metabolic control. Thus, intensive control during the original randomized phase of DCCT translated into a striking reduction in the risk of new-onset microalbuminuria over 8 years of EDIC follow-up, even though the difference in mean HBA1c between the two former treatment groups at EDIC baseline (7.4 versus 9.1%) nearly disappeared during the subsequent follow-up. The long-lasting benefit of early intensive metabolic control, or 'metabolic memory', underscores that optimizing metabolic control as soon as the diagnosis of diabetes is established may translate into long-term clinical benefits that will never be achieved if optimal intervention is started later in the history of disease.

Type 2 diabetes

Intensive glycaemic control has been shown to also benefit patients with type 2 diabetes. Renal outcome analyses of the United Kingdom Prospective Diabetes Study (UKPDS33), a trial of 3867 newly diagnosed patients with type 2 diabetes, found that intensive control (achieved HBA1c of 7%) compared to standard control (achieved HBA1c of 7.9%) decreased the risk of new-onset microalbuminuria by 24% at 9 years (UK Prospective Diabetes Study Group, 1998). This benefit, although significant, was smaller than the one observed in patients with type 1 diabetes in the DCCT, a finding that might be partly explained by the smaller difference in achieved HBA1c between the intensive and standard groups in the UKPDS (0.9%) than in the DCCT (1.8%). The smaller benefit of blood glucose reduction in type 2 diabetic patients could be taken to suggest that factors other than hyperglycaemia, such as hypertension, insulin resistance, or obesity, far more prominent in type 2 than in type 1 diabetics, might have an even stronger effect on the onset of microalbuminuria.

Importantly, comparable findings from the Kumamoto Study (Ohkubo et al., 1995; Shichiri et al., 2000), wherein intensive glycaemic control reduced the rate of new-onset microalbuminuria, converge to indicate that reducing HBA1c to 7% or less is also renoprotective in type 2 diabetes.

Recent trials have been designed to evaluate whether longer protective effects against the onset of microalbuminuria could be achieved by an even more intensified metabolic intervention. In 11,140 type 2 diabetics of the ADVANCE trial, 5 years of intensive metabolic control reduced new-onset microalbuminuria by 9% compared to standard control (Patel et al., 2008), while intensive versus standard control reduced the onset of microalbuminuria by 15% in 10,251 type 2 diabetics from the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial over 3.5 years of follow-up (Ismail-Beigi et al., 2010). The reasons for the above differences remain largely elusive since patient characteristics (including diabetes duration) and HBA1c concentrations achieved in the intensive and standard groups (6.5 vs 7.3% in ACCORD; 6.4 vs 7.5% in ADVANCE) were quite similar. Independent of the above, strict metabolic control did not translate into a reduced cardiovascular risk in both studies, and was also associated with an increased risk of all-cause and cardiovascular mortality in ACCORD, for reasons that remain debated (Dluhy and McMahon, 2008; Lachin, 2010; Riddle, 2010). Based on the above, current guidelines generally consider a target of 6.5–7% to be more appropriate than tighter control, until further evidence is met (National Institute for Health and Care Excellence, 2009). Actually, the HbA1c target of 6.5% or less may be appropriate for further reducing diabetes-related microvascular complications—including new-onset microalbuminuria—in selected individual patients, if it can be achieved without significant hypoglycaemia (American Diabetes Association, 2012a), but may be harmful in patients with longstanding diabetes and high risk of cardiovascular disease.

Blood pressure reduction

Evidence that blood pressure reduction is *per se* an intervention for preventing new-onset microalbuminuria in diabetic patients is essentially derived from the UKPDS 38 (UKPDS Study Group, 1998) and the Appropriate Blood Pressure in Diabetes (ABCD) trials, both in type 2 diabetics (Estacio et al., 2000; Schrier et al. 2002). A reasonable explanation for the lack of studies on blood pressure control in patients with type 1 diabetes is that at the stage of normoalbuminuria only a small proportion of this population is also hypertensive. When the UKPDS study started enrolling patients in the 1970s, blood pressure values < 180/105 mmHg were considered by many as an acceptable target in diabetic patients. As a consequence, at the start of the hypertension study in 1987, over 1000 patients with type 2 diabetes were randomly assigned to what at that time was considered an 'intensive' control (blood pressure < 150/85 mmHg) or to 'standard' control (blood pressure < 180/105 mmHg). Achieved blood pressure values were 144/82 and 154/87 mmHg in the 'intensive' and the 'standard' groups, respectively, and more intensified blood pressure control was eventually associated with a remarkable protective effect against both micro- and macrovascular complications over a median follow-up of 8.4 years. Of note, each 10-mmHg decrease in mean systolic blood pressure was associated with a 12% reduction in risk for all complications related to diabetes and with an 11% reduction of risk of myocardial infarction. When considering the progression from normo- to microalbuminuria, 'tight' blood pressure control reduced the risk by a striking 29% compared to 'standard' control (UKPDS Study Group, 1998). Two years after the impressive results of the UKPDS 38 had become available, the results of the ABCD trial were also published. ABCD was undertaken to investigate whether a further reduction of blood pressure target would have allowed additional benefits on diabetes-related microvascular complications, GFR decline, and cardiovascular events in patients with type 2 diabetes. To test the above hypothesis, patients were divided into two cohorts, depending upon the presence of a baseline

diastolic blood pressure > 90 mmHg (hypertensive cohort) (Estacio et al., 2000) or in the range 80–90 mmHg (normotensive cohort) (Schrier et al. 2002). In the hypertensive cohort, 470 patients were randomized to either intensive or standard blood pressure control (diastolic goal of 75 mmHg or 80–90 mmHg, respectively) (Estacio et al., 2000). Within each group, patients were further randomized to receive either nisoldipine or enalapril as the primary antihypertensive medication. Achieved blood pressure was 132/78 and 138/86 mmHg in the intensive and the standard groups, respectively, and mean renal function (assessed by 24-hour creatinine clearance) remained stable in both groups throughout the observation period. Intensive blood pressure control compared to standard control did not significantly reduce the rate of progression from normo- to microalbuminuria (25% vs 18%, respectively) or the risk of any cardiovascular outcomes, including cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, or heart failure hospitalizations, over 5 years of follow-up. However, tight blood pressure control allowed a significant reduction of all-cause mortality, from 10.7% in the standard control group to 5.5% in the intensive arm. This beneficial effect was noted in the absence of any differences between treatment groups in other risk factors, including smoking prevalence and lipid or blood glucose levels. It is also worth mentioning that the ABCD Data and Safety Monitoring Committee halted the comparison between nisoldipine and enalapril after 4 years of follow-up because much fewer myocardial infarctions occurred in patients randomly allocated to enalapril. Therefore, the Committee recommended switching the nisoldipine-treated patients to enalapril therapy. In the cohort of normotensive patients (N = 480), the goal of intensive blood pressure control was to reduce diastolic blood pressure by 10 mmHg using either nisoldipine or enalapril, while patients in the control group received a placebo. Over 5 years of follow-up, achieved blood pressure was 128/75 and 137/81 mmHg in the intensive and standard control groups, respectively. As also observed in the hypertensive cohort, mean renal function of patients with normoalbuminuria at baseline remained stable in both groups over the 5 years of follow-up. On the other hand, progression from normo- to microalbuminuria was markedly slower in the intensive than in the standard control group (17 vs 28%, P = 0.012); moreover, 15 patients reverted from micro- to normoalbuminuria in the intensive group compared to eight patients in the standard group (P = 0.03). These findings are in sharp contrast to those in patients from the hypertensive cohort, wherein intensive blood pressure control was not associated with any appreciable benefit on the progression from normo- to microalbuminuria. Actually, it is not a matter of blood pressure control, since the magnitude of the difference in achieved systolic and diastolic blood pressure between the two groups (about 8 mmHg) was very similar in both the hypertensive- and normotensive cohorts. Second, the degree of glycaemic control, although poor, was comparable between the two groups of both cohorts throughout the follow-up period. Whether the above findings reflect random data fluctuations or may have a specific, systematic explanation remains elusive. Thus, further randomized trials evaluating the differential effects of distinct blood pressure targets in patients with type 2 diabetes are clearly needed to shed some light on this grey area.

RAAS inhibition

Pivotal studies by Brenner's group in Boston in the 1980s showed that ACEI therapy started at the induction of diabetes almost completely prevented the functional and structural changes of experimental DN in rats (Zatz et al., 1986). Later studies, particularly in experimental models of streptozocin-induced diabetes (Perico et al., 1994), consistently showed that response to RAAS inhibition was a function of time, as proteinuria reduction and prevention of glomerulosclerosis were quite common when treatment was started early after the induction of diabetes; conversely, when RAAS inhibition was started after changes associated with DN were already established, the antiproteinuric and renoprotective effects were less consistent (Perico et al., 1994). These findings provided the background for testing the efficacy of early intervention with RAAS inhibitors in human disease.

Type 1 diabetes

Pioneering clinical studies from the groups of Passa (Passa et al., 1987), Pedersen (Pedersen et al., 1988), and Wiegmann (Wiegmann et al., 1992) in the late 1980s and early 1990s suggested that ACEI treatment may reduce the progression to microalbuminuria in patients with type 1 diabetes, normoalbuminuria, and hypertension (Passa et al., 1987) or even normal blood pressure (Pedersen et al., 1988; Wiegmann et al., 1992). The above findings, however, were unreliable because studies were all underpowered and flawed by major limitations. To address this important issue, investigators of the EURODIAB controlled trial of Lisinopril in Insulin-Dependent diabetes (EUCLID) study group carried out a randomized, controlled trial in 530 normotensive patients with type 1 diabetes (83% normoalbuminuric) who were randomly assigned to the ACEI lisinopril or placebo. At 2 years, albuminuria was 2.2 micrograms/min lower in the treatment than in the placebo arm, although this difference was almost fully explained by treatment effect in the minority of patients with microalbuminuria at baseline (EUCLID Study Group, 1997). Ten years later, similar findings were reported in two large placebo-controlled studies, DIRECT-Prevent 1 and DIRECT-Protect 1, evaluating the effects of the ARB candesartan versus placebo in 3326 patients with type 1 diabetes, normoalbuminuria, and normal blood pressure followed for a median of 4.7 years (Chaturvedi et al., 2008). Participants without retinopathy were recruited to the DIRECT-Prevent 1 and those with retinopathy to the DIRECT-Protect 1. Although candesartan reduced the incidence of retinopathy (but not its progression), it did not provide any protective effects against new-onset microalbuminuria. The Renin Angiotensin System Study (RASS)—a 5-year trial of 285 normotensive type 1 diabetics randomly assigned to the ACEI enalapril, the ARB losartan, or placebo—showed that enalapril did not significantly lower the incidence of microalbuminuria compared to placebo, while losartan was actually associated with a threefold increase in the risk of microalbuminuria compared to placebo (Mauer et al., 2009). Neither enalapril nor losartan achieved any beneficial effects on the fraction of glomerular volume occupied by mesangium (primary outcome of the trial), measured to evaluate the progression of renal changes in very early stages of DN or other histological features of DN at 5-year biopsy evaluation compared to baseline. Thus, available evidence does not support the concept that RAAS blocking agents may slow the progression from normo- to microalbuminuria in low-risk normotensive subjects with type 1 diabetes. However, it is plausible that studies on RAAS blockade in patients with type 1 diabetes with normal blood pressure and low vascular risk probably require much longer follow-up to establish whether it may have a role in the primary prevention of cardiorenal

complications (Bilous et al., 2009). No data are available in patients with type 1 diabetes and hypertension.

Type 2 diabetes

While the above studies in patients with type 1 diabetes focused on subjects with normal blood pressure, studies on progression from normo- to microalbuminuria in patients with type 2 diabetes largely included hypertensive patients (Table 149.4).

In 1992, Chan and co-workers found that all 18 patients with type 2 diabetes, hypertension, and normoalbuminuria who received the ACEI enalapril did not progress to microalbuminuria over 1 year of follow-up, as compared with two of the 23 patients receiving nifedipine (Chan et al., 1992). Although these encouraging results did not reach formal statistical significance, this study paved the way for continued efforts to study the favourable effects of RAAS blocking agents in normoalbuminuric patients with type 2 diabetes. The subsequent Japan Multicenter Investigation of antihypertensive treatment for Nephropathy in Diabetics (J-MIND) found a lower incidence of microalbuminuria in 95 patients with type 2 diabetes, hypertension, and normoalbuminuria randomly allocated to 2 years of treatment with enalapril than in 106 controls allocated to the dihydropyridine CCB nifedipine (15.8% versus 21.7 %) (Baba, 2001). Although suggestive, these findings were again inconclusive as the study was underpowered to detect a specific treatment effect on new-onset microalbuminuria. Moreover, both the above studies evaluated the effects of RAAS inhibition versus those of a dihydropyridine CCB, thus it was not possible to assess whether differences between treatment arms were explained by a protective effect of enalapril or rather by a harmful effect of nifedipine. The BENEDICT study was the first large-scale, prospective clinical trial designed to formally assess whether persistent microalbuminuria (defined as UAER in the microalbuminuric range, confirmed by at least two of three consecutive overnight urine collections) may be prevented in patients with type 2 diabetes, hypertension, and normal urinary albumin excretion at inclusion (UAER < 20 micrograms/min) (Ruggenenti et al., 2004). Overall, 1204 patients were randomized to the ACEI trandolapril alone, the non-dihydropyridine CCB verapamil alone, trandolapril plus verapamil, or placebo. Verapamil was also tested in the trial because of previous evidence from small studies that non-dihydropyridine CCBs could reduce proteinuria in patients with type 2 diabetes and macroalbuminuria (Bakris et al., 1996; Bakris et al., 1997). Over a median follow-up of 3.6 years, microalbuminuria developed in 5.7% of patients on combined therapy, 6% of those on trandolapril alone, 11.9% of those on verapamil alone, and 10% of those on placebo. Thus, trandolapril halved the risk of new-onset microalbuminuria compared to non-ACEI therapy. This significant effect was not enhanced by add-on non-dihydropyridine CCB. On the other hand, the effect of non-dihydropyridine CCB therapy was similar to that of placebo. The reduced incidence of microalbuminuria associated with trandolapril therapy (alone or combined with verapamil) was significant even after adjustment for baseline and follow-up systolic and diastolic blood pressure, which indicated that ACE inhibition had a specific renoprotective effect that could not be explained by

Table 149.4 Main randomized controlled trials with ACEIs or ARBs in patients with type 1 or type 2 diabetes and normoalbuminuria

Study name (reference)	Year	Study population	RAAS inhibitor agent	Renal endpoint	Patients with endpoint/total patients (%)		Hazard ratio of endpoint with use of RAAS inhibitor (95% CI)	P
					Treatment group	Placebo group		
EUCLID (EUCLID Study Group, 1997)	1997	Type 1 diabetes, normotensives	Lisinopril	AER ≥ 20 mcg/min and < 200 mcg/min	13/213 (6%)	18/227 (8%)	1.30 (0.64–2.7)	0.5
BENEDICT (Ruggenenti et al., 2004)	2004	Type 2 diabetes, hypertensives	Trandolapril	AER ≥ 20 mcg/min and < 200 mcg/min	18/301 (6%)	30/300 (10%)	0.47 (0.26–0.83)	0.01
DIRECT-Prevent 1 (Chaturvedi et al., 2008)	2008	Type 1 diabetes, normotensives	Candesartan	AER ≥ 20 mcg/min and < 200 mcg/min	NA	NA	1.08 (0.54–2.19)	NA
DIRECT-Protect 1 (Chaturvedi et al., 2008)	2008	Type 1 diabetes, normotensives	Candesartan	AER ≥ 20 mcg/min and < 200 mcg/min	NA	NA	1.03 (0.72–1.46)	NA
RASS (Mauer et al., 2009)	2009	Type 1 diabetes, normotensives	Enalapril	AER ≥ 20 mcg/min and < 200 mcg/min	4/94 (4%)	6/95 (6%)	NA	0.96
RASS (Mauer et al., 2009)	2009	Type 1 diabetes, normotensives	Losartan	AER ≥ 20 mcg/min and < 200 mcg/min	16/94 (17%)	6/95 (6%)	NA	0.01
ROADMAP (Haller et al., 2011)	2011	Type 2 diabetes, hypertensives	Olmesartan	Urinary ACR > 25 (men) or > 35 mg/g (women)	178/2160 (8.2%)	210/2139 (9.8%)	0.77 (0.63–0.94)	0.01

ACR = albumin:creatinine ratio; AER = albumin excretion rate; NA = not applicable; RAAS = renin–angiotensin–aldosterone system.

differences in blood pressure control between treatment groups (Ruggenenti et al., 2004). When outcomes were analysed according to achieved systolic (< or > 139 mmHg) blood pressure (139 mmHg was the median value achieved in whole study groups during the follow-up period (Ruggenenti et al., 2006)), the risk of progression to microalbuminuria was found to be significantly higher in those with higher blood pressure with a trandolapril effect found to be remarkably higher in this population rather than in patients with better blood pressure control on follow-up (Ruggenenti et al., 2004, 2006). Because the large majority of patients with diabetes do not achieve effective control of systolic blood pressure in clinical practice, even with the administration of two or more antihypertensive drugs, these findings highlight the importance of including ACEIs in their antihypertensive regimen because of their additional, specific renoprotective effect. Again, therapy with non-dihydropyridine CCB was ineffective at any level of achieved blood pressure.

After the BENEDICT trial, a virtually identical study, the Randomised Olmesartan And Diabetes MicroAlbuminuria Prevention (ROADMAP) study, was designed to assess whether the ARB olmesartan could reduce the incidence of microalbuminuria compared to placebo in 4400 subjects with type 2 diabetes and normoalbuminuria (Haller et al., 2011). Over 2000 patients were randomized to non-RAAS inhibitor therapy, despite previous evidence from BENEDICT showing that ACE inhibition was protective against the development of microalbuminuria (Ruggenenti et al., 2004). Over 3.2 years of follow-up, microalbuminuria developed in 8.2% of the subjects in the olmesartan group versus 9.8% in the placebo group, an effect that was observed in parallel with better blood pressure control in the olmesartan group and that was no longer significant after adjustment for achieved blood pressure values in the two treatment arms. Overall, in the BENEDICT and ROADMAP trials, trandolapril and olmesartan reduced the hazard for microalbuminuria by 56% and 23% versus placebo, respectively. As cumulative incidence of microalbuminuria was nearly identical in the placebo arms of both studies, the larger renoprotective effect of trandolapril could not be explained by different patient risk for considered events (Fig. 149.8).

Of interest, the overall rates of cardio- and cerebrovascular events—about 2.9 cases per 1000 person-years—were also similar in the control groups of both trials. However, whereas in BENEDICT the number of events was reduced by trandolapril compared to placebo (no statistics were feasible because of the small number of events), an opposite treatment effect was observed in ROADMAP, with a statistically significant fivefold increase in the risk of death from cardiovascular causes in the olmesartan group which was mainly seen in patients with history of cardiovascular disease. Whether excess cardiovascular risk while on olmesartan therapy can be explained by a specific effect of the study drug or rather by larger blood pressure reduction achieved by olmesartan, resulting in a possible J-curve effect (Cooper-DeHoff et al., 2010; Redon et al., 2012) in patients with pre-existing coronary heart disease, deserves further scrutiny. Independent of the above, after reviewing the results of the ROADMAP and ORIENT trials (Imai et al., 2011), the US Food and Drug Administration (FDA) has determined that olmesartan is not recommended as a treatment to prevent or delay microalbuminuria in diabetic patients (Food and Drug Administration, 2011).

The favourable effects of ACEIs on preventing the progression from normo- to microalbuminuria have been recently confirmed by the Action in Diabetes and Vascular disease: PreterAx and DiamicroN modified release Controlled Evaluation (ADVANCE) trial, which assessed the effects on micro- or macrovascular disease of a fixed combination of the ACEI perindopril and the diuretic indapamide in 11,140 patients with type 2 diabetes and at least one additional risk factor, who had a wide range of blood pressure values at inclusion, that averaged 145/81 mmHg (Patel et al., 2007). Over 4.3 years of follow-up, a significant reduction in the onset of microalbuminuria was observed in the perindopril-indapamide arm compared to placebo (relative risk (RR) reduction: 21%). However, findings that active therapy reduced systolic and diastolic blood pressure by 5.6 and 2.2 mmHg, respectively, compared to placebo, were inconclusive in warranting a specific treatment effect or a blood pressure reduction-related benefit. Moreover, the comparison between a fixed combination of an ACEI plus a diuretic versus placebo did not allow the treatment effect of one component of the combination to be separated from that of the other one, this being a major limitation of studies evaluating treatment effect of fixed combinations versus placebo.

Based on the encouraging results of the BENEDICT trial, the Prospective, Randomized, Probe Trial to Evaluate Whether, at Comparable Blood Pressure Control, Combined Therapy With the ACEI Benazepril and the ARB Valsartan, Reduces the Incidence

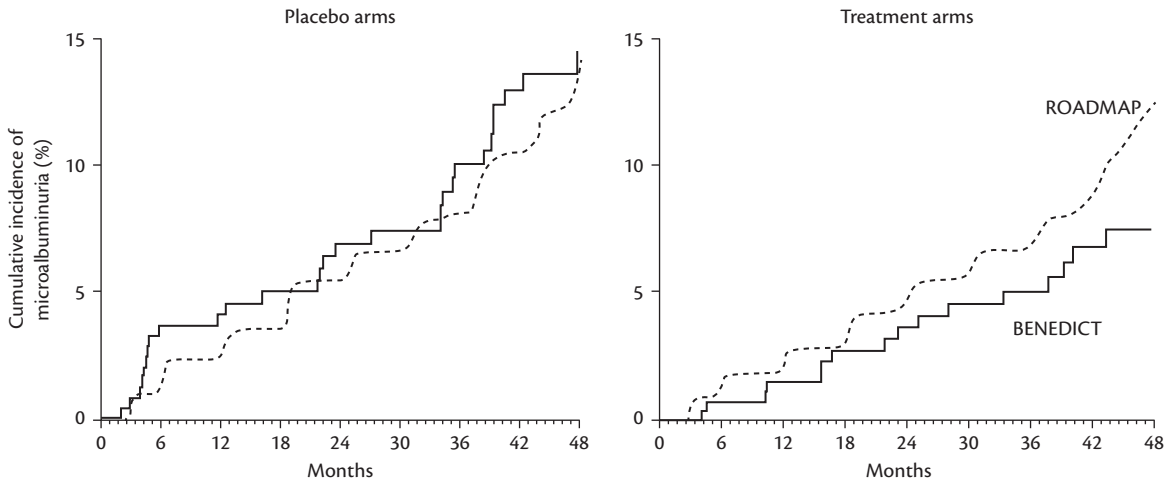

Fig. 149.8 Cumulative incidence of microalbuminuria in the placebo (left panel) and treatment arms (right panel) of the BENEDICT and ROADMAP studies.

of Microalbuminuria More Effectively Than BEN or VAL Alone in Hypertensive Patients With Type 2 Diabetes and High-normal Albuminuria (VARIETY; NCT00503152, <http://clinicaltrials.gov/ct2/show/NCT00503152>) trial was designed and is currently ongoing to assess whether the protective effect of ACEI therapy against the development of microalbuminuria in hypertensive patients with type 2 diabetes at increased risk, owing to high-normal albuminuria at inclusion, is also shared by ARB therapy and is even enhanced when ACEIs and ARBs are administered in combination. To avoid the confounding effect of larger blood pressure reduction on dual RAAS blockade, ACEIs and ARBs are administered at halved doses in the combination therapy group.

Preventing macroalbuminuria in patients with microalbuminuria

Metabolic control

Type 1 diabetes

In type 1 diabetes, preliminary positive findings from some Scandinavian studies (Feldt-Rasmussen et al., 1986; Beck-Nielsen et al., 1990; Dahl-Jorgensen et al., 1992; Bangstad et al., 1994; Parving, 1998) were not confirmed by the DCCT (The DCCT Study Group, 1993, 1995) or the Microalbuminuria Collaborative Study (Microalbuminuria Collaborative Study Group, United Kingdom, 1995). In the DCCT, there was no difference in the number of patients who progressed from micro- to macroalbuminuria between the intensive-control and the standard therapy group. Of note, however, only 10 out of the 73 originally microalbuminuric patients had progressed to overt nephropathy at the end of the observation period, which reduced the power of statistical analyses comparing the effects of the two study treatments (The DCCT Study Group, 1995). Of note, the benefits of intensive glucose control clearly emerged during the subsequent EDIC study. Indeed, this study showed that the risk of progression to overt nephropathy was reduced by 84% in patients previously assigned to the intensive control group compared to those originally in the standard control group (The EDIC Study Group, 2003). In the Microalbuminuria Collaborative Study, 70 normotensive patients with type 1 diabetes and microalbuminuria were randomly allocated to 'intensive' metabolic control or 'standard' control. Over 5 years of follow-up, six patients in each group progressed to clinical albuminuria. Based on data from the DCCT and EDIC studies, it is plausible that the observation period of the Collaboration study was too short to adequately test the potential benefits of initial treatment in this population. Therefore, based on EDIC findings, early intensified metabolic control is recommended in daily clinical practice to protect patients with microalbuminuria from progression to overt nephropathy.

Type 2 diabetes

Over 8 years of follow-up, intensive metabolic control reduced the incidence of overt nephropathy (16% vs 40%) compared to standard control in normotensive, microalbuminuric type 2 diabetics included in the secondary-intervention cohort of the Kumamoto trial (Ohkubo et al., 1995; Shichiri et al., 2000). Along the same line, 5-year intensive glycaemic control was associated with significantly slower progression to macroalbuminuria (2.9 vs 4.1%) compared to less intensive control in type 2 diabetics of the ADVANCE trial (Patel et al., 2008). A similar trend, although not significant, towards a reduced risk of progression to macroalbuminuria was observed in 1791 military veterans with type 2 diabetes from the Veterans Affairs Diabetes Trial (VADT), who were randomized to either intensive metabolic control or standard control (7.6% vs 12.1%, P = 0.1) (Duckworth et al., 2009). Interestingly, in the latter study, intensive blood glucose control allowed a statistically significant reduction in the risk of progressing from normo- to micro-, and then macroalbuminuria compared to standard control over 5 years of follow-up (2.9 vs 5.1%, P = 0.04). A recent meta-analysis of seven trials including 28,065 patients with type 2 diabetes, followed for 2–15 years, confirmed that intensive glucose control significantly reduced the risk of progression to macroalbuminuria compared to less intensive control by 26% (Coca et al., 2012). Treatment effect was significant and the finding that, at meta-regression analysis, larger differences in HBA1c between intensive and conventional therapy at the study level associated with greater benefits, provided consistent evidence of a specific protective effect of intensified metabolic control against progression from micro- to macroalbuminuria. On the other hand, the same meta-analysis failed to detect any protective effect of intensive glucose control on the risk for hard renal outcomes, including doubling of serum creatinine, ESRD, or death from renal causes.

Blood pressure targets

Existing trials on the role of different blood pressure targets on the progression from micro- to macroalbuminuria have been exclusively conducted in patients with type 2 diabetes. In the UKPDS 38, 'intensive' blood pressure control was associated with a non-significant 39% reduction in the risk of progression to macroalbuminuria at 6 years (UKPDS Study Group, 1998). On the other hand, intensive blood pressure control halved the risk of overt nephropathy (18% vs 37%) in the normotensive cohort of the ABCD trial, an effect that was statistically significant (P = 0.02) (Schrier et al., 2002). However, no benefit was observed in the hypertensive cohort of the same study (Estacio et al., 2000). Thus, whether intensified blood pressure control, without increasing the doses of RAAS inhibitors, is renoprotective *per se* in this context is uncertain.

RAAS inhibition

Type 1 diabetes

The European Microalbuminuria Captopril Study (Viberti et al., 1994) and the North American Microalbuminuria Study (Laffel et al., 1995) showed that ACEI therapy significantly reduced progression to persistent macroalbuminuria and preserved renal function compared to placebo in normotensive patients with type 1 diabetes, microalbuminuria, and normal renal function. These findings were confirmed and extended by a meta-analysis of 12 trials in 698 patients with type 1 diabetes and microalbuminuria followed for at least 1 year showing that ACEIs reduced the incidence of macroalbuminuria by 62% and increased the rate of regression to microalbuminuria by threefold compared to placebo (The ACE Inhibitors in Diabetic Nephropathy Trialist Group, 2001). Of note, Parving and Hovind found that the prevention of macroalbuminuria was also associated with a stabilization of kidney function for as long as 8 years (Parving and Hovind, 2002).

Type 2 diabetes

The beneficial effects of ACEIs in patients with type 2 diabetes and microalbuminuria were firstly demonstrated by Ravid and

co-workers in 1996 in a randomized trial showing that only 12% of the normotensive patients in the ACEI group developed overt nephropathy compared to 42% of those in the placebo group. Of note, albumin excretion rate remained stable in enalapril-treated patients over 7 years of follow-up (Ravid et al., 1996). A few years later, these pioneering findings were confirmed and extended by the larger Heart Outcomes Prevention Evaluation (HOPE) study, a randomized clinical trial aimed at evaluating the cardioprotective effects of a 5-year treatment with the ACEI ramipril compared to placebo in > 9000 patients at increased risk of cardiovascular events (Yusuf et al., 2000). In the subgroup of 3577 patients with type 2 diabetes, those on ramipril had a significantly lower incidence (24%) of progression to macroalbuminuria compared to controls (HOPE Study Investigators, 2000). This effect was associated with a 22% decrease in the incidence of myocardial infarction, stroke, or death from cardiovascular causes, despite a similar degree of blood pressure control in the two treatment arms. The first formal demonstration that overt DN can be prevented in type 2 diabetes, however, was provided by the IRbesartan in patients with type 2 diabetes and MicroAlbuminuria (IRMA 2) study, a randomized clinical trial involving 590 hypertensive diabetics with microalbuminuria (Parving et al., 2001). The study found that 2 years of treatment with irbesartan 300 mg daily achieved a threefold reduction in the incidence of macroalbuminuria compared to placebo, while a lower dose (150 mg/day) was remarkably less effective (Parving et al., 2001). Consistently, the Incipient to Overt: Angiotensin II Blocker, Telmisartan, Investigation on Type 2 Diabetic Nephropathy (INNOVATION) study (Makino et al., 2007) found that in 527 Japanese subjects with type 2 diabetes and microalbuminuria randomized to telmisartan (40 or 80 mg) or placebo, 16.7% progressed to overt nephropathy in the 80 mg arm compared to 22.6% in the 40 mg arm and 49.9% in the placebo arm over 1.3 years of follow-up, a risk reduction that was highly significant in both telmisartan arms versus placebo. Of note, a lower rate of progression toward overt nephropathy was also observed in a subgroup of 163 normotensive patients, suggesting that the renoprotective effects of telmisartan were not fully explained by blood pressure lowering per se. The rates of reversion to normoalbuminuria (21.2% and 2.8% of patients treated with telmisartan 80 and 40 mg, respectively) were similar to those observed in the IRMA 2 study with two different doses of irbesartan.

The BENEDICT-B trial found that, in patients with type 2 diabetes and microalbuminuria, ACEI therapy with trandolapril associated with rates of progression to macroalbuminuria similar to those achieved by full-dose irbesartan in IRMA 2, an effect that was not affected by combined treatment with verapamil. Of note, however, over the 4-year observation period approximately 50% of patients regressed from micro- to normoalbuminuria, an effect that was associated with a halved risk of fatal and non-fatal cardiovascular events (9.8% in regressors vs 18.9% in non-regressors) (Ruggenenti et al., 2011a). Blood pressure was comparable in both groups and ACEI therapy was identical. Thus, the observed differences in albuminuria and cardiovascular event rates between the two groups must necessarily reflect intrinsic patient characteristics that may affect their responsiveness to ACE inhibition.

Head-to-head comparisons

ACEIs versus ARBs

The Diabetics Exposed to Telmisartan and Enalapril (DETAIL) study, the only head-to-head, long-term comparison of ACE inhibition and ARB therapy in diabetic patients with renal involvement, was designed to demonstrate that the renoprotective effects of the ARB telmisartan (80 mg/day) were not inferior to those of enalapril (10 mg/day) in type 2 diabetic patients with micro- or macroalbuminuria and hypertension (Barnett et al., 2004). The primary efficacy variable was the change in the GFR, directly measured by iohexol plasma clearance technique, over 5 years of follow-up. Since at final analyses the lower boundary of the difference in GFR between the two treatment groups (−7.6 mL/min/1.73 m^2, in favour of enalapril) was greater than the predefined value of −10 mL/min/1.73 m^2, the study authors concluded that telmisartan did not appear to be less effective than enalapril. However, GFR reduction at 5 years was 20% larger in the telmisartan group and 27 out of the 120 patients on telmisartan (22.5%) had fatal or non-fatal cardiovascular events compared to 21 out of the 130 on enalapril (16.1%). Unfortunately, no statistics were provided to ascertain whether the above differences were significant. Independent of the above, considering the daily costs of the two medications (0.125 euros for enalapril vs 1.026 for telmisartan), the cost-effectiveness of enalapril is largely superior to that of telmisartan, at least in this context.

RAAS blocking agents versus CCBs

CCBs differ in their effect on glomerular haemodynamics and UAER (Smith et al., 1998). Conventional dihydropyridine CCBs may induce vasodilation of afferent renal arterioles with little changes in the efferent arteriole diameter. At a given systemic blood pressure, the predominant pre-glomerular vasodilation results in increased glomerular perfusion pressure and increased intraglomerular pressure, that may impair, at least in theory, the sieving function of the glomerular filtration barrier (Bakris et al., 2004). Third-generation dihydropyridine CCBs, such as manidipine, barnidipine, and lercanidipine, may induce vasodilation in both the afferent and the efferent arteriole. This results in increased perfusion but at lower glomerular pressure, an effect that, in theory, should translate into improved glomerular sieving function (Hayashi et al., 1996, 2003, 2007). Non-dihydropyridine CCBs (verapamil, diltiazem) may have renal haemodynamic effects similar to those of RAAS inhibitors, and small, older studies suggested that these agents could reduce proteinuria in patients with type 2 diabetes and nephropathy (Harris et al., 2006). Despite the above differences, the observations on the differential benefits of dihydropyridine and non-dihydropyridine CCBs are conflicting and of uncertain clinical relevance (Griffin et al., 1999).

One of the first trials comparing the nephroprotective effects of ACEIs and dihydropyridine CCBs was published by Mimran and co-workers in 1988 (Mimran et al., 1988). In this study, which enrolled patients with type 2 diabetes, normal blood pressure, and microalbuminuria, first-generation dihydropyridine CCB nifedipine was found to associate with increases in UAER, while the ACEI captopril resulted in opposite effects (Mimran et al., 1988). In a subsequent study by Velussi and co-workers, 26 and 18 hypertensive patients with type 2 diabetes with normo- or microalbuminuria, respectively, were randomly assigned to the ACEI cilazapril or the second-generation CCB amlodipine (Velussi et al., 1996). In the microalbuminuric subgroup, mean yearly GFR decline was similar in both treatment arms (2.15 ± 0.69 vs 2.33 ± 0.83 mL/min/1.73 m^2, respectively). In addition, cilazapril and amlodipine lowered AER to a similar extent in both normo- and

microalbuminuric patients. A subsequent trial showed that the ACEI lisinopril and the first-generation dihydropyridine CCB nifedipine were similarly effective in delaying the occurrence of overt proteinuria in 92 normotensive patients with type 1 diabetes and microalbuminuria followed for 3 years (Crepaldi et al., 1998). Overall, the above studies were too small to provide any conclusive results on the antiproteinuric and renal effects of CCBs versus RAAS inhibitor therapy.

Two remarkable larger trials better clarified this issue. Third-generation dihydropyridine CCB lercanidipine and enalapril achieved a similar reduction in AER in 277 patients with type 2 diabetes, hypertension, and persistent microalbuminuria from the DIAL (Diabete, Ipertensione, Albuminuria, Lercanidipina) trial over 9–12 months of follow-up (Dalla Vestra et al., 2004). ARBs and dihydropyridine CCBs were compared in the MicroAlbuminuria Reduction With VALsartan (MARVAL) study, wherein 332 patients with type 2 diabetes and microalbuminuria were randomized to either the ARB valsartan or the CCB amlodipine (Viberti et al., 2002). After 24 weeks, the UAER was 56% of baseline in the valsartan arm and 92% of baseline in the amlodipine arm ($P < 0.001$), despite a similar reduction in blood pressure values (systolic/diastolic: −11.2/6.6 mm Hg for valsartan, −11.6/6.5 mm Hg for amlodipine). In addition, reversion to normoalbuminuria was more frequent with valsartan than amlodipine (29.9% of patients versus 14.5%, $P = 0.001$). Thus, the above two trials confirmed that third-generation dihydropyridine CCBs may actually have an antiproteinuric effect similar to that of RAAS inhibitors, an effect that is not shared by older dihydropyridine CCBs. Whether this antiproteinuric effect may also translate into significant nephroprotection is unknown.

Combination therapies based on RAAS blocking agents

When high blood pressure associates with high glucose circulating levels, which almost invariably occurs in type 2 diabetes, in particular in the presence of renal involvement, these two risk factors synergistically interact to further accelerate end-organ damage and increase the risk of stroke, coronary heart disease, left ventricular hypertrophy, and ESRD. Thus, optimized control of diabetes and hypertension is of paramount importance in this population. Achieving blood pressure targets < 130/80 mmHg, however, is extremely difficult in type 2 diabetes, and blood pressure control is seldom on target, even when two or more blood pressure-lowering medications are used in combination. Poor compliance, in addition to intrinsic resistance to treatment, in particular in those with evidence of renal involvement, are the most frequent causes of treatment failure in this population. To address this issue, blood pressure-lowering combinations have been introduced in clinical use. Combination therapy may help in achieving a tighter blood pressure control with less risk of adverse effects, by using relatively small doses of two drugs in combination or selecting agents that counteract each other's side effects (Prisant et al., 2003). A comprehensive analysis of 354 randomized trials of the five main categories of blood pressure-lowering drugs highlighted that, unlike the antihypertensive effects of drugs in combination, the prevalence of adverse effects is not additive (Law et al., 2003). In particular, single agents caused symptoms in 5.2% of participants compared to 7.5% of those who received combination treatment, which is significantly lower than the value of 10.4% with an additive effect. Target blood pressure is also achieved more rapidly and with less adverse events with fixed combinations compared to higher-dose monotherapy (Taylor, 2004). Fixed-dose combination therapy may also simplify the treatment regimen, thus improving compliance and preventing treatment failures caused by missed doses (Epstein and Bakris, 1996) and may also allow cost reductions to the healthcare systems (Taylor, 2004). On the other hand, fixed-dose combinations do not allow easy dose adjustment (Williams et al., 2005; Frank, 2008). ACEI plus ARB, or ACEI/ARB plus diuretic or CCB have been so far the regimens most frequently tested in patients with type 2 diabetes.

ACEIs plus ARBs

Given the superior antiproteinuric effect of dual RAAS blockade compared to either drug alone (Kunz et al., 2008), treatment with both ACEIs and ARBs might be theoretically advisable in most patients with DN (Ruggenenti et al., 2009). In fact, the combination of ACEIs and ARBs inhibits the RAAS more effectively than either agent alone (Jacobsen et al., 2003b). In patients receiving treatment with ACEIs, add-on ARB therapy inhibits the activity of angiotensin II produced via ACE-independent pathways, while in patients on ARB therapy the addition of ACE inhibition might limit compensatory angiotensin production induced by AT_1 receptor blockade. The above interactions probably explain why combined therapy with an ACEI and an ARB decreased albuminuria significantly more than single-drug RAAS blockade in patients with type 1 diabetes and nephropathy (Jacobsen et al., 2002, 2003a). In the CAndesartan and Lisinopril Microalbuminuria (CALM) study (Mogensen et al., 2000), which randomized 199 hypertensive patients with type 2 diabetes and microalbuminuria to 12 weeks of monotherapy with candesartan or lisinopril, followed by 12 weeks of monotherapy or combination treatment, albuminuria decreased significantly more with combined treatment than with candesartan or lisinopril alone.

In the above-mentioned studies, however, dual RAAS blockade was associated with a superior antihypertensive effect compared with monotherapy. Thus, whether the superior antiproteinuric effect was the result of more effective RAAS inhibition with combination therapy or was just the consequence of more effective blood pressure reduction remained elusive. This issue was addressed in a crossover study that compared the antiproteinuric effect of fixed doses of benazepril and valsartan given alone with the antiproteinuric effect of halved doses of the two drugs given in combination to patients with non-diabetic CKD (Campbell et al., 2003). Dual therapy was associated with the greatest reduction in proteinuria. Blood pressure, however, was similarly reduced with each of the three treatments, indicating that the superior antiproteinuric effect of dual over single-drug RAAS blockade was actually due to more effective RAAS inhibition (Campbell et al., 2003). Unpublished studies support these observations.

RAAS blocking agents plus diuretics

The rationale of ACEI/ARB plus diuretic combinations is based on evidence that ACEIs and ARBs, in addition to counterbalance diuretic-induced RAAS activation, or hypokalaemia/hypomagnesaemia, may have their antiproteinuric effect amplified by concomitant diuretic therapy, particularly in patients with high sodium intake (Reboldi et al., 2009). Indeed, sodium intake may blunt the antiproteinuric effect of RAAS blocking agents (Vegter et al., 2012), and the concomitant use of thiazide diuretics may overcome

this blunting effect (Buter et al., 1998). As salt sensitivity is more common in African Americans than in white people (Gibbs et al., 1999), RAAS blocking agents plus diuretics may be especially useful, wherein monotherapy with conventional doses of ACEIs or ARBs is often unsuccessful or marginally successful (Weinberger et al., 1985). Finally, ACEIs or ARBs may mitigate diuretic-induced alterations in glucose metabolism (Opie, 1999; Izzo et al., 2011). The advantages of combined therapy versus ACE inhibition monotherapy were confirmed by the Preterax in Albuminuria Regression (PREMIER), a trial of 457 patients with type 2 diabetes, hypertension, and microalbuminuria randomized to perindopril plus indapamide (N = 233) or enalapril alone (N = 224) and followed for 52 weeks (Mogensen et al., 2003). Perindopril plus indapamide significantly reduced AER (by 42 versus 27%) and the rate of serious cardiovascular events (2.5 versus 6.3%) compared to ACEI alone, while tolerability was comparable between the two treatment arms. An obvious limitation of the above study is that the ACEI used as monotherapy was different from that used for the fixed combination, thus the theoretical possibility exists that differences between treatment groups could be explained by different effects of the two ACEIs, for instance because of the use of non-equivalent doses. A nearly significant reduction in new or worsening nephropathy was also observed in the perindopril-indapamide group of the ADVANCE trial, compared to placebo (RR reduction: 18%; P = 0.055) (Patel et al., 2007).

RAAS blocking agents plus CCBs

The combination of a RAAS blocking agent plus a CCB may provide consistent additional blood pressure reduction and, in some circumstances, additional favourable effects on glucose metabolism, possibly mediated by improved insulin sensitivity, on top of the well-known benefits of RAAS inhibitor therapy (Ferrier et al., 1991; Rubio-Guerra et al., 2004). First, CCBs may produce vasodilation, thus enhancing skeletal muscle blood flow, insulin and glucose delivery, and non-oxidative pathways of glucose utilization. Second, CCBs may also improve insulin sensitivity at the cellular level by decreasing the cytosolic-free calcium concentrations (Baron et al., 1988; Draznin et al., 1988). RAAS blockade may also counterbalance CCB-induced renin release, thus enhancing the antihypertensive effects of CCBs (Cheng and Frishman, 1998), and allow additional benefits on left ventricular hypertrophy, fibrinolytic balance, and arterial distensibility (Weir, 2007).

The renal- and cardioprotective effects of RAAS blocking agents plus CCBs have been tested in a number of clinical trials. In 309 patients with type 2 diabetes, hypertension, and microalbuminuria randomly assigned to the dihydropyridinic CCB amlodipine, the ACEI fosinopril, or both medications, combination treatment achieved a larger reduction in blood pressure values and AER compared to either drug alone over 4 years of follow-up (Fogari et al., 2002). In addition, regression from micro- to normoalbuminuria at 4 years was achieved in 67% of patients on combined therapy, a rate that significantly exceeded the 33% and 46% incidence of regression observed with amlodipine or fosinopril monotherapy, respectively. These findings were not confirmed by subgroup analyses from the Study of Hypertension and the Efficacy of Lotrel in Diabetes (SHIELD), showing that benazepril plus amlodipine versus benazepril alone did not achieve any additional benefit on blood pressure control or AER reduction over 12 weeks of follow-up (Winer et al., 2005). This substudy, however, was clearly underpowered to test treatment effect on considered outcomes, since only 20 patients were included. None of the above studies explored the effects of ACEIs plus CCBs on renal disease progression. To address this issue, the Delapril and Manidipine for Nephroprotection in Diabetes (DEMAND) trial (Ruggenenti et al., 2011b) evaluated the rate of GFR decline, measured by iohexol plasma clearance, in 380 hypertensive patients with type 2 diabetes and normo- or microalbuminuria, randomly allocated to delapril plus manidipine, delapril alone, or placebo. Data showed that the monthly rate of GFR decline was similar on combined therapy (0.32 mL/min/1.73 m^2), delapril (0.36 mL/min/1.73 m^2), or placebo (0.30 mL/min/1.73 m^2). Independent of the above findings, this trial provided the novel information that, even before the onset of overt nephropathy, type 2 diabetics have a rate of renal function loss that exceeds by three to six times the rate reported in healthy subjects. Failure to slow GFR decline by any considered treatment compared to placebo highlighted the need for early intervention with novel treatments targeting potential mediators of accelerated renal function loss already at the stage of normo- or microalbuminuria (Ruggenenti et al., 2011b). On the other hand, combination therapy safely reduced cardiovascular events, ameliorated retinopathy and neuropathy, and also limited the worsening of insulin resistance over time. Overall, the above trials provided evidence that RAAS inhibitor plus CCB combinations may offer renal advantages compared to either drug alone. Only one study, however, evaluated the effects of different doses of the individual components of the combination in 300 patients with type 2 diabetes, hypertension, and microalbuminuria, initially treated with telmisartan 40 mg/day plus amlodipine 2.5 mg/day (Fogari et al., 2007), then randomized to two-dose titration regimens, one based on increasing doses of telmisartan (up to 160 mg daily) and a fixed dose of amlodipine (2.5 mg/day), the other one based on increasing doses of amlodipine (up to 10 mg daily) and a fixed dose of telmisartan (40 mg/day). Data showed that, at similar levels of blood pressure reduction, a greater decrease in AER was evident in subjects treated with increasing doses of telmisartan.

More recently, RAAS inhibitor-based combinations, including either a diuretic or a CCB, have been evaluated in head-to-head comparisons in hypertensive type 2 diabetics with microalbuminuria. In the Gauging Albuminuria Reduction with Lotrel in Diabetic Patients with Hypertension (GUARD) trial (Bakris et al., 2008b), 1-year benazepril/amlodipine and benazepril/hydrochlorothiazide (HCTZ) combinations achieved a similar reduction in urinary ACR compared to baseline and similar rates of progression to overt DN. However, while benazepril plus amlodipine achieved a larger reduction in blood pressure values, initial treatment with benazepril plus HCTZ resulted in a greater reduction in albuminuria. On the other hand, available data suggest that third-generation CCBs may have an antiproteinuric effect similar to that of ACEIs and ARBs. For instance, in the Add-on manidipine versus amlodipine in diabetic patients with hypertension and microalbuminuria (AMANDHA) trial (Martinez-Martin et al., 2008), manidipine on top of full-dose treatment with RAAS inhibitors reduced the AER by 65.5% versus 20% with amlodipine add-on. Thus, the addition of manidipine resulted in a larger reduction in the UAER compared to amlodipine despite similar blood pressure reductions. The different antiproteinuric effects of second- and third-generation dihydropyridine CCBs may also explain the striking difference

between the GUARD trial, wherein benazepril plus HCTZ allowed a greater reduction in AER than benazepril plus amlodipine, and another recently published trial in hypertensive type 2 diabetics, showing that third-generation dihydropyridine CCB manidipine on top of the ARB candesartan reduced the urinary albumin excretion by 53%, while thiazide diuretic add-on was ineffective (Fogari et al., 2007). The novel concept of a potential superiority of RAAS blocking agents plus CCBs versus RAAS inhibitors plus diuretics has also been suggested and expanded to cardiovascular outcomes by the publication of additional data from the Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension (ACCOMPLISH) trial (Bakris et al., 2010; Weber et al., 2010), showing that in patients at high risk for cardiovascular events and with different degrees of albuminuria, the ACEI benazepril plus the dihydropyridine CCB amlodipine almost halved the incidence of the renal endpoint of 50% or more increase in serum creatinine and significantly reduced the first occurrence of a cardiovascular event or death from cardiovascular causes compared to benazepril plus HCTZ. The cardiovascular benefits of benazepril plus amlodipine were particularly evident in a subgroup of high-risk diabetics with history of cardiovascular or renal events, independent of haemodynamic effects. However, the hypothesis that a RAAS blocking agent, combined with a second- or third-generation CCB rather than HCTZ, may be the first-choice combination in high-risk diabetic patients, such as those with renal involvement, deserves further scrutiny. This is especially true for renal endpoints observed in ACCOMPLISH, as benazepril plus HCTZ induced an acute haemodynamic change in GFR within 12 weeks, while benazepril plus amlodipine slightly increased GFR. However, the subsequent long-term slope was similar between the two treatment groups. Moreover, the composite renal endpoint in ACCOMPLISH (Bakris et al., 2010) was completely driven by the doubling of serum creatinine, which might reflect a reversible haemodynamic change in GFR, with no difference in the rate of ESRD (Heerspink et al., 2010).

Preventing or slowing progression to ESRD

Protein restriction

More than 60 years ago, Addis hypothesized that renal disease progression could be ameliorated by reducing the burden of excretory nitrogen through dietary protein restriction (Addis, 1948). Micropuncture studies in the 1980s actually found that dietary protein restriction abrogates the adaptive rise in glomerular pressure, thereby slowing the tendency to disease progression in hyperfiltering kidneys of rats with reduced nephron mass (Hostetter et al., 1981) as well as in experimental diabetes (Zatz et al., 1985) and mineral-corticoid induced hypertension (Dworkin et al., 1984). Results of dietary manipulation studies in humans, in whom blood pressure tends to be well controlled and ACE inhibitors used, have not been so clear-cut. This is discussed further in Chapter 101.

Thus, whether and to what extent protein intake restriction should be recommended to slow progression in type 2 diabetic patients with advanced renal disease is still uncertain. Conceivably, a controlled protein intake approximating 0.8 g/kg body weight/day could be suggested, combined with a low-salt intake (Vegter et al., 2012), to avoid the side effects of nitrogenous waste products without increasing protein catabolism.

Metabolic control

While the pivotal role of metabolic control in preventing the onset and progression of DN in subjects with normo- or microalbuminuria is now well established (Coca et al., 2012), the impact of glycaemic control on progression from macroalbuminuria to ESRD is less clear. The beneficial influence of intensive metabolic control in patients with type 1 (Hovind et al., 2001; Rossing et al., 2002) or type 2 diabetes (Rossing et al., 2004) is suggested by observational studies, but evidence from prospective intervention studies has been scant. In 2011, De Boer and co-workers evaluated time-dependent changes in estimated (by the CKD-EPI equation) GFR in a large cohort of patients with type 1 diabetes included in the DCCT trial and subsequently monitored in the EDIC study (Perkins, 2012). They found that intensive metabolic control compared to conventional therapy reduced the risk of impaired GFR (defined as eGFR < 60 mL/min/1.72 m^2) and the combined outcome of impaired GFR or death. Intensive metabolic control was associated with faster GFR decline during DCCT, but with slower decline during the EDIC study. On the basis of these findings, the study authors concluded that in patients with type 1 diabetes, early glycaemic control may help in preventing renal function impairment in the long term. Unfortunately, intensive glycaemic control did not achieve a statistically significant reduction of ESRD compared to standard control. Along the same line, intensive glucose control did not reduce the risk of doubling of serum creatinine (RR 1.06, 95% confidence interval (CI) 0.92–1.22) or death from renal disease in a recent systematic review of seven trials involving 28,065 patients with type 2 diabetes (Coca et al., 2012). There was a non-significant trend towards reduction of ESRD, that apparently contrasts with the null findings for the endpoints of doubling of serum creatinine or death from renal disease. However, the absolute rate of clinical renal outcomes in the included studies was relatively low, and the pooled cumulative incidence of doubling of serum creatinine and ESRD was only 4.1% and 1.6%, respectively, in the standard treatment group of all trials. In light of the low incidence of these endpoints, intensive glucose control could not be justified given the risks of hypoglycaemia, the minimal impact on cardiovascular outcomes, and the potential increased risk of death (Gerstein et al., 2008). Several reasons may explain the discouraging results of the meta-analysis. It is likely that *early* intensive metabolic control is necessary to improve long-term renal outcomes of diabetic subjects, while a late start of intensive control is unable to halt the progression of diabetic kidney disease. It is also possible that the duration or the magnitude of intensive glucose control may have been inadequate to reduce progression. In conclusion, the meta-analysis of Coca and co-workers underscores that intensive metabolic control does not reduce the risk of doubling of serum creatinine or death from renal disease in patients with DN; in addition, intensive glucose control would reduce the absolute risk of ESRD of < 1% in the best-case scenario. Thus, available evidence does not support the use of intensive glycaemic control for preventing renal failure in diabetic patients with macroalbuminuria.

Blood pressure reduction

It is well established that RAAS inhibitors have a specific renoprotective effect in patients with type 2 diabetes and overt DN, independent of blood pressure control (Brenner et al., 2001; Lewis et al., 2001). However, whether RAAS inhibition can be

enhanced by intensified blood pressure control in this population is unclear. In patients with type 1 diabetes and DN, who had previously participated in the Angiotensin-Converting Enzyme Inhibition in Diabetic Nephropathy Study, intensive blood pressure control, targeting mean arterial pressure (MAP) < 92 mmHg (with more intense RAAS inhibition), decreased proteinuria and reduced GFR progressive deterioration more effectively than standard blood pressure control (MAP of > 100 to < 107 mmHg) (Lewis et al., 1999), suggesting that intensive blood pressure control combined to ACEI therapy not only contributed to arrest renal disease progression, but also provided clinical evidence of regression/remission of renal disease. However, whether the treatment effect was explained by intensified blood pressure control or rather by more effective RAAS inhibition, or both, cannot be definitely established.

The role of intensive blood pressure control in patients with type 2 diabetes and overt nephropathy has been evaluated in a predefined *post hoc* analysis of the Reduction of Endpoints in Non-insulin-dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) study (Bakris et al., 2003). In this study, a systolic blood pressure of < 130 mmHg was associated with a significant reduction in the risk of the composite primary endpoint of doubling of serum creatinine, ESRD or death when compared with systolic blood pressure values > 140 mmHg. Similarly, diastolic blood pressure < 70 mmHg were associated with a reduced risk of reaching the primary endpoint when compared to diastolic blood pressure > 90 mmHg. It is worth mentioning, however, that intensive blood pressure control was not associated with any appreciable benefit on hard renal outcomes in patients with non-diabetic nephropathies from the MDRD, AASK, and REIN-II trials (Klahr et al., 1994; Wright et al., 2002; Ruggenenti et al., 2005).

RAAS inhibition

In both type 1 and type 2 diabetes, RAAS inhibitor therapy is invariably associated with a significant reduction in urinary protein excretion that translates into significant renoprotection (The ACE Inhibitors in Diabetic Nephropathy Trialist Group, 2001; Remuzzi et al., 2002b). The antiproteinuric effect is largely mediated by an improved sieving function of the glomerular barrier that translates into reduced protein traffic and nephrotoxicity (Remuzzi et al., 2006; Cravedi et al., 2012). Further, the improved glomerular sieving function also results in an improved selectivity of proteinuria with a remarkably reduced excretion of proteins larger than albumin (Fig. 149.9, left panel), a finding explained by improved size-selective function of the glomerular barrier (Fig. 149.9, right panel).

Type 1 diabetes

A pioneering study published by Taguma and co-workers in 1985 (Taguma et al., 1985) showed that captopril at doses that did not affect systemic blood pressure allowed the regression of nephrotic syndrome in 10 uraemic patients with overt DN. In the late 1980s and early 1990s, randomized clinical studies from Scandinavian groups (Parving et al., 1988; Bjorck et al., 1992) showed that GFR decline was slower in patients with type 1 diabetes on ACEI therapy than controls on non-ACEI therapy. In 1993, the Collaborative Study (Lewis et al., 1993) found that 3 years of captopril treatment were associated with a blood-pressure independent 50% reduction of the combined endpoint of doubling of serum creatinine, ESRD or death

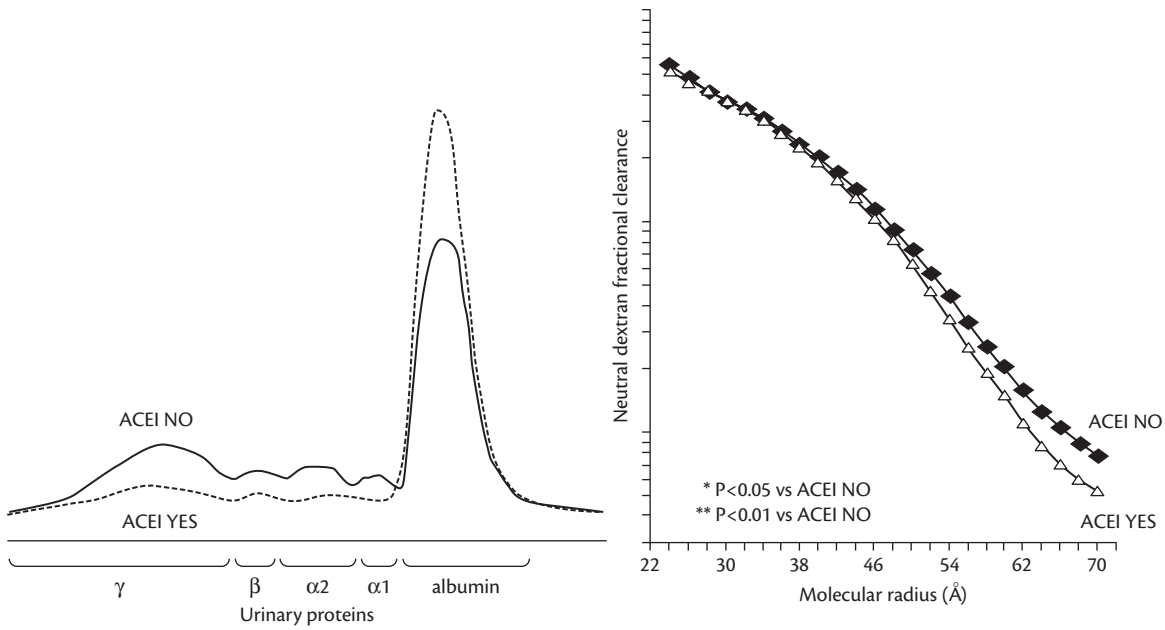

Fig. 149.9 Left panel: urinary protein electrophoresis in a subject with proteinuric nephropathy before (ACEI NO) and after (ACEI YES) ACEI treatment. ACEI therapy reduced 24-hour proteinuria from 900 to 280 mg and improved urinary protein selectivity, as shown by the relative increase in urinary albumin compared to other urinary proteins. Right panel: Fractional clearances of neutral dextrans in 24 patients with proteinuric nephropathy before (ACEI NO) and after (ACEI YES) ACEI therapy. ACEI therapy reduced proteinuria and improved the size selective function of the glomerular barrier.

compared to placebo in 409 patients with type 1 diabetes, albuminuria > 500 mg per day and serum creatinine < 221 micromol/L (< 2.5 mg/dL). Captopril therapy also halved the risk of cardiovascular mortality, which provided the first evidence that ACEIs have a specific cardioprotective effect in patients with diabetes.

Type 2 diabetes

Approximately 10 years later, the RENAAL trial (Brenner et al., 2001) and the Irbesartan in Diabetic Nephropathy Trial (IDNT) (Lewis et al., 2001) showed that ARB therapy versus placebo decreased the incidence of the composite endpoint of doubling of serum creatinine concentration, ESRD, or death by 16% and 20%, respectively, in two large cohorts of patients with type 2 diabetes and overt nephropathy. Again, renoprotection was associated with a significant reduction in urinary protein excretion, that remained significant even after adjustment for the small differences in blood pressure control between treatment groups. Independent of treatment allocation, both trials showed that early reduction in urinary protein excretion was associated with a slower decline of renal function in the long term.

Of note, however, treatment effect on considered endpoints was remarkably smaller in RENAAL (Brenner et al., 2001) and IDNT (Lewis et al., 2001) than in the Collaborative Study Group (Lewis et al., 1993), despite patients from the above studies having similar renal function and proteinuria at the beginning of the study (Table 149.5).

Whether the different effect might reflect a different response to therapy in patients with type 2 or type 1 diabetes with overt nephropathy or rather a larger efficacy of ACEIs on considered outcomes is matter of speculation.

The above studies also provided important insights on the cardioprotective effects of RAAS blocking agents in patients with type 2 diabetes and overt nephropathy, showing that early reduction in albuminuria was also associated with less long-term cardiovascular mortality (de Zeeuw et al., 2004). A recent post hoc analysis of the combined data from the RENAAL and the IDNT trials suggests that albuminuria and blood pressure may represent independent targets for cardioprotective therapy in this population (Holtkamp et al., 2011). RAAS inhibitors were associated with a variable and discordant reduction in systolic blood pressure and UAE, and 34.5% of patients either had a reduction in systolic blood pressure but no reduction in albuminuria, or vice versa, indicating a substantial discordance in response in these parameters. Of note, the initial reduction in systolic blood pressure or albuminuria—from baseline to 6 months—independently correlated with cardiovascular protection. After 6 months, a progressively lower cardiovascular risk was observed with lower UAE, across all categories of systolic blood pressure. These data suggest the importance of titrating treatment not only to blood pressure, but also to urinary albumin, in order maximize renal and cardiovascular benefits.

Head-to-head comparisons

Comparisons between different ARBs

The A comparison of telMisartan versus losArtan in hypertensive type 2 DiabEtic patients with Overt nephropathy (AMADEO) study aimed to investigate whether a highly lipophilic ARB with a long half-life, telmisartan, would offer better nephroprotection than a less lipophilic agent with a shorter half-time, losartan, in the presence of a similar degree of blood pressure control (Bakris et al., 2008a). Eight hundred and sixty patients with type 2 diabetes, hypertension, and overt nephropathy were randomized to either telmisartan (40–80 mg/day) or losartan (50–100 mg/day) and followed for 52 weeks. The primary endpoint was the difference in urinary ACR at 52 weeks. Telmisartan achieved a greater reduction in albuminuria than losartan (−29.8% vs −21.4%). In contrast to these findings, however, the inVestIgate the efficacy of telmisartan versus VALsartan in hypertensive type 2 DIabetic patients with overt nephropathy (VIVALDI) study (Galle et al., 2008) did not find any differences in proteinuria reduction between telmisartan and valsartan in 885 hypertensive patients with type 2 diabetes and overt nephropathy. The above apparent inconsistency was likely explained by the fact that a non-significant trend for lower systolic blood pressure values (about 2 mmHg) was evident in the telmisartan group of the AMADEO trial, while blood pressure control was similar between the two groups of the VIVALDI study. Evidence that even small differences in blood pressure control may result in significant differences in cardiovascular events supports that the above finding could actually be explained by the observed difference in systolic blood pressure (Verdecchia et al., 2010). Alternatively,

Table 149.5 Main randomized controlled trials with ACEIs or ARBs in patients with type 1 or type 2 diabetes and overt nephropathy.

Study name (reference)	Year	Study population	Study drug	Primary endpoint	Patients with endpoint/total patients (%)		Hazard ratio of endpoint with use of RAAS inhibitor (95% CI)	P
					Treatment group	Placebo group		
Collaborative Study Group (Lewis et al., 1993)	1993	Type 1 diabetes	Captopril	Doubling of serum creatinine	25/207 (12%)	43/202 (21%)	0.52 (0.16–0.67)	0.007
RENAAL (Brenner et al., 2001)	2001	Type 2 diabetes	Losartan	Doubling of serum creatinine, ESRD, or death	327/751 (44%)	359/762 (47%)	0.84 (0.72–0.98)	0.02
IDNT (Lewis et al., 2001)	2001	Type 2 diabetes	Irbesartan	Doubling of serum creatinine, ESRD, or death	189/579 (33%)	222/569 (39%)	0.81 (0.67–0.99)	0.03

RSRD = end-stage renal disease; RAAS = renin–angiotensin–aldosterone system.

telmisartan and valsartan are similarly effective on considered outcomes, whereas losartan has a lower antiproteinuric effect.

RAAS blocking agents versus other drugs

Twenty-three years ago, 30 patients with diabetes and overt proteinuria were randomized to either enalapril 40 mg/day or nifedipine 40 mg/day (Ferder et al., 1992). All patients were on hypoproteic diet (0.8 g/kg/day). After 1 year of treatment, mean proteinuria significantly fell from 4.36 to 0.56 g/24 hours in the enalapril group, but did not change in the nifedipine group. Furthermore, creatinine clearance remained stable in the enalapril group, but fell significantly from 67.8 mL/min to 51.4 mL/min in patients treated with nifedipine. Similar findings on proteinuria were observed in a subsequent open-label study in patients with type 2 diabetes and overt nephropathy randomized to the ARB losartan (N = 44) or the dihydropyridine CCB amlodipine (N = 43) for a 12-week titration phase followed by a 12-week maintenance phase. Although both drugs significantly reduced blood pressure values, only losartan significantly decreased 24-hour UAER (from 810 to 570 mg/day), while proteinuria did not change in the amlodipine group (Yasuda et al., 2005). Consistently, ACEIs generally exerted superior antiproteinuric action also in head-to-head comparisons with beta-blockers (Nielsen et al., 1994).

The specific renoprotective effects of ACEIs and ARBs were evaluated by a meta-analysis of trials comparing renal outcomes in heterogeneous populations of patients who had been randomized to RAAS inhibition or other antihypertensive agents (Casas et al., 2005). Pooled results from small studies showed that RAAS blockade had beneficial effects on renal outcomes in high-risk patients with diabetic or non-diabetic chronic nephropathies; by contrast, pooled results from large cardiovascular trials showed no or small benefit of RAAS blockade on renal outcomes in low-risk patients with no evidence of renal disease. Even with the above limitations, however, overall data showed that ACEIs or ARBs significantly lowered the risk of ESRD by 13% and the risk of a doubling of serum creatinine by 29% compared to other antihypertensive drugs. This risk reduction could not be explained by differences in blood pressure control, and its magnitude was similar to that observed for cardiovascular events (Remuzzi and Ruggenenti, 2006). The finding that ACEIs and ARBs reduced albuminuria more effectively than non-RAAS-inhibitor therapy in almost 5000 patients with diabetes, without differences in systolic and diastolic blood pressure between treatment groups and independent of albuminuria levels at inclusion, provided additional evidence of the specific antiproteinuric and renoprotective effect of those medications (Casas et al., 2005).

ACEIs plus ARBs

Dual RAAS inhibition using maximally tolerated doses of ACEIs and ARBs combined with intensified blood pressure, metabolic, and lipid control (the 'Remission Clinic' approach) achieved the remission or regression of proteinuria (< 0.3 or greater or equal to 0.3 and < 1 g/24 hours, respectively) and stabilized or improved the GFR in 26 of 56 non-diabetic patients who had nephrotic-range proteinuria refractory to treatment with conventional antihypertensive doses of an ACEI (Ruggenenti et al., 2008b). Further reduction of proteinuria and slower GFR decline were also achieved in non-diabetic patients without remission or regression, even if treatment effect was smaller in this subgroup. Over 4 years of follow-up, only 2 of the 56 patients treated according to the Remission Clinic protocol progressed to ESRD compared with 17 of 56 reference patients matched by gender, age, and urinary protein excretion and receiving conventional ACEI therapy (Ruggenenti et al., 2008b). The renoprotective effect associated with the Remission Clinic approach was modest, however, in patients with type 2 diabetes. Despite use of intensified and multidrug treatment, follow-up systolic blood pressure largely exceeded the target in most patients, which might, at least in part, have offset the antiproteinuric and renoprotective effect of RAAS inhibition in this population.

This is consistent with the findings of a recent trial in patients with type 2 diabetes and advanced DN and poor metabolic control, showing that combination treatment with enalapril plus losartan did not achieve any additional benefits on proteinuria compared to enalapril alone and did not affect urinary inflammatory cytokines (MCP-1, TGF-beta, and VEGF) (Titan et al., 2011). In the recently published Olmesartan Reducing Incidence of End Stage Renal Disease in Diabetic Nephropathy Trial (ORIENT) study (Imai et al., 2011), 577 patients (377 Japanese, 200 Chinese) with type 2 diabetes and overt nephropathy were randomized to either the ARB olmesartan or placebo. The primary outcome was a composite of doubling of serum creatinine, ESRD, chronic dialysis, transplantation and death. Only 70% of included patients were already treated with RAAS inhibitor therapy, despite consolidated evidence that RAAS inhibitors have a significant renoprotective effect in this population. After 3.2 years of follow-up, similar number of patients in the olmesartan and placebo group (hazard ratio (HR) 0.97, 95% CI 0.75–1.24, P = 0.791) progressed to the primary composite outcome showing no effect of the intervention on this population. Of interest, cardiovascular deaths were higher in the olmesartan than in the placebo arm (10 vs 3 cases), despite larger blood pressure and proteinuria reduction in the olmesartan group (major adverse cardiovascular events and all-cause deaths were similar between the two groups). As expected, hyperkalaemia was more common in the olmesartan than the placebo group (9.2% vs 5.3%). The ORIENT study had several limitations. In particular, there was a strong imbalance of pre-existing cardiovascular disease between the olmesartan and placebo arms, as about twice as many patients assigned to the olmesartan group had a history of cardiovascular disease than in the placebo group, which might have partly explained the excess incidence of cardiovascular events in the olmesartan group.

The recently published VA NEPHRON D trial (Fried et al., 2013), which enrolled 1448 subjects with type 2 diabetes and overt proteinuria in spite of full-dose ARB therapy, investigated the effects of dual RAAS blockade compared to ARB monotherapy on a primary composite endpoint of changes in eGFR, ESRD, or death and a secondary endpoint of reduction in eGFR or ESRD. Study data showed a non-significant trend toward a benefit from dual blockade on both the primary (HR 0.88; 95% CI 0.70–1.12; P = 0.3) and the secondary end point (HR 0.78; 95% CI 0.58–1.05, P = 0.10). On the other hand, combination treatment versus single RAAS blockade increased the risk of hyperkalemia or acute kidney injury, which caused the early termination of the trial after only 2.2 years, compared to a planned follow-up time in the original protocol of up to 5 years. Such increased risk was likely due to the up-titration scheme of RAAS blocking agents, that was driven by the absence of unacceptable adverse events rather than any predefined threshold of decrease in ACR and/or BP values; in addition, no baseline screening for mono- or bilateral stenosis of the renal artery was actually performed. The early termination of the trial prevented the demonstration of a clear-cut relationship between dual RAAS

blockade and a lower risk of ESRD, that nevertheless nearly reached formal statistical significance (HR 0.66, i.e. a 34% risk reduction; P = 0.07).

Two ongoing randomized trials, VALID and VARIETY (ClinicalTrials.gov registry numbers NCT00494715, <http://clinicaltrials.gov/ct2/show/NCT00494715> and NCT00503152, <http://clinicaltrials.gov/ct2/show/NCT00503152>, respectively), will formally address whether dual RAAS blockade results in more efficient protection against progression to ESRD than single-drug RAAS inhibition in patients with type 2 diabetes and overt nephropathy (VALID) or against the development of microalbuminuria or CV events in high-risk patients with type 2 diabetes and normoalbuminuria (VARIETY).

The ONTARGET issue

Since the renoprotective effect of RAAS inhibitors is largely explained by their antiproteinuric effect, it is reasonable to predict that these drugs will not offer specific nephroprotective effects in patients with non-proteinuric renal disease (Maschio et al., 1996; Locatelli et al., 1997; Ruggenenti and Remuzzi, 2009). Actually, those patients would be unnecessarily exposed to the risks associated with RAAS inhibition, which include hyperkalaemia and acute renal function deterioration (Ruggenenti et al., 2009); such events are particularly common in elderly individuals and in patients with type 2 diabetes and decreased GFR and/or renal vascular disease (Takaichi et al., 2007). The risks of these adverse effects are even increased when RAAS inhibition is maximized with combined ACE inhibition and ARB therapy, as shown by the ONgoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial (ONTARGET) (Mann et al., 2008). This study randomly assigned 25,620 patients with established atherosclerotic vascular disease, including 6982 individuals with diabetes and end-organ damage, to ramipril, telmisartan, or a combination of the two. Over 56 months of follow-up, the incidence of cardiovascular events was similar in the three treatment groups, whereas the pre-specified composite outcome of any dialysis, renal transplantation, a doubling of serum creatinine, or death occurred more frequently in patients on combination treatment than in those on telmisartan or ramipril alone. The finding that the increased reduction in proteinuria achieved with combined therapy was associated with an increase in the number of these adverse events has been misleadingly interpreted as a need for the reappraisal of proteinuria as a suitable surrogate endpoint for improvement in renal function in CKD patients (Epstein, 2009). In actual fact, however, the excess of adverse renal outcomes on combination treatment was significant only for the more frequent need for acute haemodialysis to treat transient kidney dysfunction in patients on combination therapy. Conceivably, this increased need for acute haemodialysis largely reflected transient kidney hypoperfusion in patients with excessive blood-pressure reduction, hypovolaemia, or ischaemic kidney disease, that improved with treatment withdrawal. Conversely, incidence of doubling of serum creatinine and ESRD events was similarly rare in all groups, reflecting the remarkably slow rate of renal function loss that, independent of treatment allocation, was close to that observed in the general population as a result of ageing (Lindeman et al., 1985). The slow rate of renal function loss can be largely explained by the fact that only 4% of patients had overt proteinuria and may also explain why RAAS inhibition did not seem to improve renal outcomes in this population. Thus, in patients at increased cardiovascular risk but without significant proteinuria, including those with nephroangiosclerosis or ischaemic kidney disease (the majority of patients with CKD), maximal RAAS inhibition does not seem to improve renal and cardiovascular outcomes and is associated with an increased risk of adverse effects. ACEIs and ARBs seem to have similar reno- and cardioprotective effects in this population that are not enhanced by their combination.

RAAS blocking agents plus CCBs

The potential benefits of combination therapy with RAAS blocking agents plus CCBs in patients with type 2 diabetes, hypertension, and overt proteinuria have been initially supported from a small and open-label study showing that verapamil plus trandolapril achieved a larger reduction in proteinuria and less adverse events compared to either drug alone or HCTZ plus guanfacine over 1 year of follow-up (Bakris et al., 1992). Similarly, verapamil plus trandolapril achieved more proteinuria reduction than either drug alone in patients with type 2 diabetes and overt nephropathy (Bakris et al., 1998). In the subgroup of patients with DN from the recent Cilnidipine versus Amlodipine Randomised Trial for Evaluation in Renal Disease (CARTER) study (Fujita et al., 2007), enrolling patients with hypertension, kidney disease, and proteinuria, already treated with background RAAS inhibitor therapy, cilnidipine achieved a greater reduction in the absolute value of urinary protein to creatinine ratio than amlodipine over 1 year of treatment.

Novel therapeutic perspectives

Direct renin inhibition

The beneficial effects of RAAS inhibition can be blunted by the increased release of renin that results from the positive feedback loop associated with the AT_1 inhibition, which occurs with ACEI or ARB therapy (Estacio, 2009). As renin catalyses the first and rate-limiting step of the RAAS cascade, inhibition of renin seems to be a sensible approach to block this loop during downstream blockade of the RAAS. Moreover, data indicate that renin inhibitors exert direct angiotensin-independent effects through prorenin receptors (Nguyen et al., 2002, 2008) expressed in the heart, liver, placenta, brain, and kidney. Overexpression of these receptors has been associated with elevated blood pressure and plasma aldosterone levels, and increased prorenin levels are associated with microvascular complications of diabetes (Estacio, 2009).

Blocking prorenin binding to the prorenin receptor has been shown to prevent renal damage in streptozotocin-induced diabetic rats (Ichihara et al., 2004). When aliskiren, a human renin inhibitor that is effective when administered orally (Eder et al., 2007), was developed for clinical use, it enabled the renoprotective effect of renin inhibition to be tested in humans with diabetic renal disease (Estacio, 2009). In 15 patients with type 2 diabetes and microalbuminuria or macroalbuminuria, treatment with aliskiren (300 mg per day) for 28 days was associated with a 44% decrease in urinary ACR and a 6–8 mmHg reduction in systolic blood pressure compared with baseline values (Estacio, 2009). A subsequent crossover study found that 2 months of treatment with aliskiren (300 mg per day) or irbesartan (300 mg per day) reduced albuminuria to a similar extent in 26 patients with type 2 diabetes and hypertension, and that use of the two drugs in combination was associated with

a greater decrease in albuminuria than use of either agent alone (Persson et al., 2009).

The Aliskiren in the eValuation of prOteinuria In Diabetes (AVOID) trial (Parving et al., 2008) was a randomized, double-blind, placebo-controlled, multinational study that aimed to evaluate the antiproteinuric effect of aliskiren (300 mg per day) in 599 hypertensive patients with type 2 diabetes and overt nephropathy on chronic treatment with losartan (100 mg per day for at least 1 month). Treatment with aliskiren was associated with a 20% reduction in albuminuria after 24 weeks of follow-up, compared with no change with placebo. Moreover, a reduction in urinary ACR of 50% or more occurred in 25% of patients on aliskiren compared with 12.5% of patients on placebo. Differences in blood pressure between treatment groups at the end of the study period were small and not significant and changes in albuminuria did not correlate with concomitant changes in arterial blood pressure. These findings suggested that aliskiren could have renoprotective effects that are independent of its blood-pressure-lowering effects in patients with hypertension, type 2 diabetes, and nephropathy (Parving et al., 2008). The above data provided the background for the ALiskiren Trial In Type 2 diabetes Using cardio-renal Disease Endpoints (ALTITUDE) study (Parving et al., 2012), that was aimed to determine whether 4-year treatment with aliskiren compared to placebo, on top of background RAAS inhibitor therapy might reduce renal and cardiovascular events in 8561 patients with type 2 diabetes, micro- and macroalbuminuria, or cardiovascular disease. Study analyses, however, failed to detect a beneficial effect of aliskiren therapy on any considered outcome and actually raised some safety concerns. At 32 months of follow-up, the primary composite endpoint (myocardial infarction, stroke, new hospitalization for heart failure, cardiovascular death, resuscitated death, ESRD or doubling of serum creatinine) occurred in 767 subjects (17.9%) in the aliskiren group and 721 (16.8%) in the placebo group (HR 1.08, 95% CI 0.98–1.20, P = 0.14). A trend towards an increased risk of stroke was evident in the aliskiren arm (HR 1.25, 95% CI 0.98–1.60, P = 0.7). Finally, while the onset of doubling of serum creatinine or ESRD was similar in the two treatment arms, aliskiren significantly increased the risk of hyperkalaemia and hypotension. For the above reasons, the study was interrupted prematurely in December 2011 on the recommendation of the data monitoring committee. Therefore, available data suggest that dual RAAS blockade with aliskiren plus ACEI or ARB has no benefit and increases the risk of adverse events, including hyperkalaemia, in patients with type 2 diabetes and/or hypertension (de Boer et al., 2012; Harel et al., 2012). Based on the above findings, aliskiren therapy is not recommended in patients with diabetes independent of the stage of their renal involvement.

Add-on aldosterone antagonism

ACEIs or ARBs significantly reduce plasma aldosterone levels, which may partly explain the systemic and renal effects of RAAS inhibition. In 50% of subjects, however, plasma aldosterone levels initially decrease, then start to increase to pre-treatment levels or even above (Rossi et al., 2006; Bomback and Klemmer, 2007). This phenomenon is known as aldosterone breakthrough, and is apparently initiated and sustained by an increased release of aldosterone in response to either increased serum potassium levels or enhanced angiotensin II production through non-ACE pathways mediated by tissue chymases and cathepsins (Rossi et al., 2006). Sodium and water retention sustained by the mineralocorticoid effects of aldosterone on the distal nephron eventually limits or blunts the blood pressure- and proteinuria-lowering effects of ACEIs or ARBs. Aldosterone might also have a synergistic effect on glomerular haemodynamics and trophic effects of angiotensin II, possibly through the upregulation of glomerular angiotensin II receptors. Finally, enhanced aldosterone levels may directly exert deleterious pro-inflammatory and pro-fibrotic effects on glomerular arterioles, podocytes, mesangial cells, and renal fibroblasts, which may contribute to the progression of kidney damage (Becker et al., 2009). Most of these effects may be abrogated by aldosterone antagonists (Struthers et al., 2008).

In salt-loaded uninephrectomized rats infused with aldosterone, heavy proteinuria and podocyte damage were abrogated by the aldosterone antagonist eplerenone, but not by the vasodilator hydralazine, despite similar blood pressure values (Du et al., 2009). Consistently, in rats with 5/6 nephrectomy receiving losartan, add-on therapy with spironolactone further reduced proteinuria and achieved regression of glomerulosclerosis, tubulointerstitial fibrosis, and vascular lesions (Piecha et al., 2008). These experimental data, along with preliminary evidence that spironolactone has an antiproteinuric effect in CKD patients treated with ACEIs (Chrysostomou and Becker, 2001), provided the rationale for a series of randomized clinical trials. These trials were performed in relatively small series of patients with proteinuric nephropathies—in most cases associated with type 2 diabetes—who were on background therapy with ACEIs, ARBs, or both. Overall, aldosterone antagonists were associated with a 30–60% reduction in albuminuria compared to placebo over 4–52 weeks of treatment (Navaneethan et al., 2009).

Consistently, in patients with type 2 diabetes, hypertension, and macroalbuminuria on lisinopril therapy, add-on therapy with the aldosterone antagonist spironolactone (25 mg/day) reduced albuminuria by 34% compared to placebo, an effect that significantly exceeded the 17% reduction observed with losartan (Mehdi et al., 2009). Albuminuria reduction seemed to reflect a specific effect of aldosterone inhibition, as blood pressure control was similar in the three treatment arms (Mehdi et al., 2009). Two studies investigating the effect of adding spironolactone to ACEI or ARB therapy found that, after an initial acceleration in renal function loss, GFR decline was slower in aldosterone-treated subjects than in controls, a finding consistent with the idea that add-on spironolactone has a renoprotective effect (Bianchi et al., 2006; van den Meiracker et al., 2006).

Despite these encouraging findings, however, adverse effects associated with the chronic administration of spironolactone, including gynaecomastia, erectile dysfunction, and menstrual abnormalities, tempered initial enthusiasms on the clinical role of this treatment. The above limitations were addressed with the development of eplerenone, a specific aldosterone antagonist that is devoid of the non-mineralocorticoid effects of spironolactone. In a randomized, multicentre trial of 286 patients with type 2 diabetes and persistent macroalbuminuria despite ACEI therapy, add-on therapy with 50 mg or 100 mg of eplerenone daily was associated with a significant reduction in urinary albumin excretion compared to placebo and the treatment was well tolerated (Epstein et al., 2006). Thus, adequately powered trials are needed to assess the long-term renoprotective effect of eplerenone and investigate whether improved renal outcomes may offset adverse effects such as hyperkalaemia, the major limitation to aldosterone-based RAAS

inhibitor therapy, particularly in patients with diabetes and renal insufficiency (Khosla et al., 2009).

PPAR-gamma agonists

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear transcription factors that modulate several physiological processes, including glucose and lipid metabolism, energy homeostasis, blood pressure, inflammation, immunity, cell proliferation, and reproduction. Among the three isotypes of PPARs, designated as PPAR-alfa, PPAR-beta/delta, and PPAR-gamma (Lehrke and Lazar, 2005), the latter has been suggested to play a major role in the pathogenesis of DN. PPAR-gamma is constitutively expressed by the kidney, mainly in the medullary collecting ducts, but also in other nephron segments, including proximal tubules, glomerular mesangial cells, podocytes, and renal vasculature (Yang et al., 2012). The possibility of a pathogenic role for PPAR-gamma is suggested by the recent observation of an association between the PPAR-2 Pro12Ala polymorphism and microalbuminuria (De Cosmo et al., 2009). This evidence provided the background for the development of novel drugs that, by exerting an agonistic activity on PPAR-gamma receptors, were expected to improve insulin sensitivity and therefore to ameliorate the consequences of insulin resistance. Among these compounds, thiazolidinediones (pioglitazone, rosiglitazone, and troglitazone) have been widely used for glucose lowering in patients with type 2 diabetes, and also achieved beneficial effects on inflammation, mesangial cell proliferation, and fibrosis in experimental models of DN (Li et al., 2006; Tang et al., 2006). The renoprotective and antiproteinuric effects of thiazolidinediones are likely mediated by multiple mechanisms (Yang et al., 2012). First, these agents may improve systemic (Ryan et al., 2011) and renal insulin resistance (Tiwari et al., 2007). Second, PPAR-gamma agonists may reduce blood pressure values through the inhibition of the angiotensin II signalling pathway and the increased synthesis of endothelial NO (Roszer and Ricote, 2010). Third, they may decrease the levels of a number of inflammatory cytokines and inhibit the renal expression of TGF-beta, which plays a pivotal role in DN (Ohtomo et al., 2007). Fourth, thiazolidinediones and other PPAR-gamma agonists, including telmisartan, may reduce the production of reactive oxygen species, that is otherwise induced by the action of advanced-glycation end products (AGEs) on their specific receptor, RAGE (Matsui et al., 2007; Matsui et al., 2010). Fifth, PPAR-gamma may activate or restore adiponectin signalling in both renal and extra-renal sites, and increased levels of adiponectin may reduce proteinuria in patients with type 2 diabetes (Miyazaki et al., 2007). Tesaglitazar, a PPARalfa/gamma dual agonist, also achieved a significant increase of adiponectin levels and a significant antiproteinuric effect in murine models (Cha et al., 2007). Finally, PPAR-gamma agonists may exert additional effects, including a reduction in glomerular and proximal tubular hypertrophy (Lai et al., 2011), the inhibition of amylin secretion (Hull et al., 2005), and the reduction of lipotoxicity in the kidney (Yang et al., 2009).

With the above background, several studies have been designed to test whether PPAR-gamma agonists, particularly thiazolidinediones, have a specific renoprotective effect in diabetic patients with evidence of renal involvement. Thirty patients with type 2 diabetes and microalbuminuria were randomly assigned to metformin (N = 13) or troglitazone (N = 17) for 12 weeks. Troglitazone significantly reduced mean ACR from 70 to 40 mg/g creatinine at 4 weeks and maintained these reduced levels for up to 8 weeks. On the other hand, metformin did not change ACR throughout the whole follow-up period (Imano et al., 1998). In a subsequent double blind, parallel-group, 32-week study, 389 subjects with type 2 diabetes and microalbuminuria were randomized to either rosiglitazone or glyburide, on top of metformin therapy (Bakris et al., 2006). Despite comparable improvements in glycaemic control in both groups, urinary ACR was significantly reduced by 22.7% in the rosiglitazone plus metformin arm, but not in the glyburide plus metformin arm. Rosiglitazone plus metformin also reduced 24-hour systolic and diastolic ambulatory blood pressure (by 3.4 and 2.9 mmHg, respectively), von Willebrand factor and fibrinogen, levels of plasminogen activator inhibitor-1 antigen and activity and C-reactive protein, compared to glyburide plus metformin. Finally, pioglitazone significantly reduced albuminuria compared to metformin in 68 patients with type 2 diabetes and microalbuminuria from the Asahikawa Prospective Pioglitazone in Microalbuminuria Effect (APRIME) study (Morikawa et al., 2011).

Despite the above encouraging findings, growing concerns about the cardiovascular safety of thiazolidinediones (Guan et al., 2005) were raised by recent studies (Nissen and Wolski, 2007, 2010). A FDA meta-analysis of randomized controlled trials (Food and Drug Administration, 2010) on PPAR-gamma agonists, found that both rosiglitazone and pioglitazone increased the risk of congestive heart failure and that the risk of all-cause mortality, cardiovascular mortality, and myocardial infarction tended to be higher with rosiglitazone than pioglitazone. The analyses, however, suffered from a number of limitations, including the low rate of events and the high heterogeneity of included studies. In order to explore the risk/benefit profile of these drugs in daily clinical practice, the UK Medicines and Healthcare products Regulatory Agency (MHRA) commissioned a study on the electronic medical records maintained by general practitioners in the United Kingdom (General Practice Research Database) (Gallagher et al., 2011). Again, a higher risk for all-cause death, cardiovascular death, and heart failure was found for rosiglitazone compared to pioglitazone, and this excess risk was largest in patients aged 65 years or older. These results are consistent with those reported by other authors using US Medicare data (Azoulay et al., 2010). On the basis of the above findings, rosiglitazone has been removed from the market in Europe and is facing restrictions in the United States.

Endothelin-receptor antagonists

Evidence of incomplete efficacy of RAAS blockade fuelled research for new drugs that target alternative pathways to reduce hypertension, proteinuria, and renal or cardiovascular events, particularly in patients with diabetes and established CKD (Ruggenenti et al., 2010c). In this perspective, the seminal discovery of the endothelial-cell derived vasopressor endothelin-1 (ET-1) (Yanagisawa et al., 1988) paved the way to experimental and clinical studies aimed to test ET-1 inhibitors on top of traditional RAAS blocking agents. ET-1 is a potent vasoconstrictor, and ET-1 antagonists have been proposed as an effective new approach to hypertension (Dhaun et al., 2008). In addition, ET-1 plays an important role in kidney injury (Remuzzi et al., 2002a). Glomerular hypertension and hyperfiltration cause a protein overload in the lumen of tubules, which induces the overexpression of ET-1, cell proliferation, and interstitial inflammation, that ultimately results in progressive kidney damage (Zoja et al., 1995). In addition, protein overload

induces podocyte expression of ET-1, which may alter glomerular permselectivity (Morigi et al., 2005). ET-1 may be antagonized by reducing its production through inhibition of ET converting enzyme (ECE), which generates the biologically active ET-1 from its precursor big ET-1 (Remuzzi et al., 2002a), or by mixed or selective inhibition of the receptors ET_AR and ET_BR.

Atrasentan, a selective ET_AR antagonist, has been recently evaluated in a randomized, double blind, placebo-controlled trial of patients with DN already treated with RAAS blocking agents (Kohan et al., 2011). Eighty-nine patients with type 2 diabetes, eGFR > 20 mL/min, and urinary ACR of 100–3000 mg/g were randomly assigned to three different doses of atrasentan (0.25, 0.75, or 1.25 mg daily) or placebo and followed for 8 weeks. An albuminuria-lowering effect was evident for the 0.75 and 1.25 mg doses throughout the study period, independent of blood pressure reduction, while the 0.25 mg dose did not allow a significant reduction in ACR compared to placebo. One serious adverse event related to fluid retention occurred in a patient with a history of cardiovascular disease, while peripheral oedema was the most common adverse event, but also relatively rare and generally mild in severity. Two additional trials investigated the effects of the ET_AR antagonist avosentan on albuminuria reduction and CKD progression (Wenzel et al., 2009; Mann et al., 2010). In the first one (Wenzel et al., 2009), 286 patients with type 2 diabetes and overt DN and blood pressure < 180/100 mmHg were randomized to avosentan (5, 10, 25, or 50 mg/daily) or placebo, on top of RAAS blocking agents. After 12 weeks of follow-up, a significant reduction in urinary ACR was evident in all four dose groups (−16.3 to −29.9%) compared to placebo. Creatinine clearance was unchanged at 12 weeks. In the second one, 1392 patients with type 2 diabetes and overt nephropathy were randomly assigned to either avosentan (25 or 50 mg) or placebo (Mann et al., 2010). The composite primary outcome was the time to doubling of serum creatinine, ESRD, or death, while secondary outcomes included changes in ACR and cardiovascular events. Although avosentan achieved a dose-dependent reduction in albuminuria by −44.3% and 49.3% with the 25- and 50-mg doses, respectively, compared to placebo, no benefits on the primary outcome were observed. An excess of cardiovascular events was also evident in patients treated with avosentan, and the study was halted prematurely. These two studies (Wenzel et al., 2009; Mann et al., 2010) highlighted the clinical relevance of fluid retention and congestive heart failure in patients treated with avosentan. Although the underlying mechanisms are not fully understood, it is possible that the relatively high doses of avosentan induced a partial blockade of the ET_BR despite its 50:1 selectivity for ET_AR to ET_BR (Neuhofer et al., 2009) and this resulted in sodium retention with secondary volume expansion. In contrast, atrasentan at relatively low doses (Kohan et al., 2011) might have achieved an incomplete blockade of ET_AR, and the consequent binding of ET-1 to both ET_AR and ET_BR, thus limiting the systemic vasodilatory effects secondary to unopposed ET-1 binding to ET_BRs.

In light of the above issues, researchers aimed to test newer endothelin-receptor inhibitors with less safety concerns related to fluid retention. Along this line, daglutril—a combined ECE and neutral endopeptidase (NEP) inhibitor, which also increases the levels of serum atrial natriuretic peptide—is particularly promising because of its natriuretic, diuretic, vasodilatory, and antiproliferative properties (Daull et al., 2007). A prospective, randomized, double-blind, crossover clinical trial evaluating the effects of daglutril on blood pressure, albuminuria, and renal function in patients with type 2 diabetes, hypertension, and residual micro- or macroalbuminuria despite best available therapy, including optimal doses of losartan, has been recently concluded (Parvanova et al., 2011).

Bardoxolone methyl

Bardoxolone methyl is an antioxidant inflammatory modulator that reversibly interacts with critical free thiol groups of cysteine residues on KEAP-1 and other target proteins, inducing the translocation of Nrf-2 to the nucleus and the activation of several genes, including glutathione S-transferase, haeme oxygenase, and other components of the cytoprotective response (Thomas et al., 2012). At high doses, bardoxolone methyl may also interact with other proteins, including PPAR-gamma, tubulin, and the kinase activator IKK. Bardoxolone methyl has been initially tested in patients with advanced refractory cancer (Tsao et al., 2010) and subsequently in subjects with type 2 diabetes and established CKD. In the Bardoxolone Methyl Treatment: Renal Function in CKD/Type 2 Diabetes (BEAM) study, 227 subjects with type 2 diabetes and eGFR between 20 and 45 mL/min/1.73 m^2 were randomly assigned to three different doses of bardoxolone methyl (25, 75, or 150 mg once daily) or placebo and followed for 52 weeks (Pergola et al., 2011). Most patients were already treated with standard therapy, including RAAS blocking agents and statins. Glycaemic (mean HBA1c at baseline: 7.2%) and blood pressure control (mean systolic blood pressure: 130 mmHg) was largely adequate. Bardoxolone methyl on top of standard therapy significantly increased eGFR by 6–10 mL/min/1.73 m^2, and the greatest benefits were observed in patients receiving 75 or 150 mg/day of the study drug. On the other hand, placebo had no effect on eGFR. However, bardoxolone methyl significantly increased adverse effects (mainly muscle spasms and nausea) in a dose-dependent fashion (Pergola et al., 2011). The study results had to be interpreted with great caution as the significant increase in eGFR with bardoxolone methyl was associated with a significant increase in albuminuria, and to date no mechanism has been suggested for the improvement in eGFR. The observed findings may reflect effects on glomerular inflammation and haemodynamics rather than any change in glomerular structure. Any treatment-induced GFR increase mediated by increased single-nephron GFR should cause concern since glomerular hyperfiltration is considered a major determinant of onset and progression of DN in experimental diabetes as well as in human disease. On the other hand, whether the increase in GFR reflects an actual increase in true GFR or rather some change in creatinine tubular handling is unknown (Ruggenenti et al., 2012c). Importantly, a major pitfall of the study is represented by the fact that GFR was not directly measured with a validated method (Gaspari et al., 1995). Concerns about the use of bardoxolone methyl in patients with DN have been recently fuelled by the early stopping of the randomized, double blind, placebo-controlled, phase 3 Bardoxolone Methyl Evaluation in Patients With Chronic Kidney Disease and Type 2 Diabetes: the Occurrence of Renal Events (BEACON) study (ClinicalTrials.gov registry number NCT01351675, <http://clinicaltrials.gov/ct2/show/record/NCT01351675>) (de Zeeuw et al., 2013). In this trial, about 1600 patients with type 2 diabetes and advanced CKD (screening eGFR between 15.0 and 30.0 mL/min/1.73 m^2) were randomized to either bardoxolone methyl 20 mg/day or placebo. The primary

endpoint was time-to-first event of a composite of ESRD (need for chronic dialysis or transplantation) and cardiovascular death. The BEACON trial was stopped on the recommendation of its independent data-monitoring committee, which reported a safety imbalance due to excess serious adverse events and mortality in the bardoxolone arm. Although the precise nature of the serious adverse events or the number of excess deaths have not yet been disclosed, the decision to stop all trials using bardoxolone in CKD patients poses a serious threat to the future of the drug. In addition, recent experimental data in Zucker diabetic fatty (ZDF) rats (Zoja et al., 2013) raised further concern about the negative side effects of bardoxolone. The rats were treated with RTA 405, a synthetic triterpenoid analogue of bardoxolone methyl, with the ACEI ramipril, or with a combination of RTA 405 and ramipril. Treatment was started at 3 months of age, when rats already exhibited proteinuria, and was continued for up to 6 months. Although ZDF rats treated with the bardoxolone analogue ate less, lost weight, and had lower blood glucose, they also had increased blood pressure values, cholesterol levels, and proteinuria, and more glomerulosclerosis compared to animals on ramipril alone (Harris, 2013). These adverse effects were not necessarily dose related, at least at the two concentrations of bardoxolone analogue used in the study, and were minimally affected by concomitant ramipril treatment. At 1 month, elevated liver enzymes were also observed in the RTA 405 group compared to placebo, but this difference was no longer evident at 3 months. However, significant increases in liver weight and marked histologic changes—hepatocyte swelling, diffuse vacuolization and cellular degeneration-point to a major RTA 405-induced liver toxicity.

Other drugs

A number of additional non-pharmacologic or pharmacologic interventions have been tested in patients with diabetes and different degrees of albuminuria, including weight reduction (Morales et al., 2003), lipid-lowering drugs (Tonolo et al., 2006; Rutter et al., 2011), pentoxifylline (McCormick et al., 2008), fenofibrate (Ansquer et al., 2005), fish oil (Rossing et al., 1996a), sulodexide (Heerspink et al., 2008; Packham et al., 2012), protein kinase C inhibitors (Tuttle et al., 2005), dipeptidyl peptidase-4 inhibitors (Hattori, 2011), antifibrotic agents (Sharma et al., 2011), urotensin receptor antagonists (Vogt et al., 2010), and pyridoxamine dihydrochloride (Lewis et al., 2012). To date, insufficient evidence exists to recommend any of these interventions for the prevention or treatment of DN, although weight loss and aggressive lipid lowering represent an important part of the medical treatment of all diabetic subjects *per se*.

Conclusions

DN will remain a challenge for both physicians and healthcare systems over the next decades, particularly in newly industrialized countries, such as China and India, and developing countries (van Dieren et al., 2010). New treatment options have arisen from experimental studies, but the number of novel molecules with nephroprotective effects implemented in the clinical practice over the last two decades is disappointingly low. Thus, new diagnostic tools and therapeutic interventions are urgently needed to fight this dreadful complication. Recent trials showed that existing drugs do not significantly improve the slope of GFR decline in diabetic patients with normo- or microalbuminuria (Ruggenenti et al., 2011b), although they may actually improve cardiovascular outcomes (Ruggenenti et al., 2011a). Thus, early detection of diabetic subjects who are prone to develop micro- or macroalbuminuria might potentially represent a suitable strategy to achieve timely therapeutic intervention and reduce the burden of diabetes-related ESRD worldwide. In this respect, recent evidence highlighted the potential usefulness of a urinary proteomic-based risk score classifier in predicting the development and progression of microalbuminuria in diabetic patients (Roscioni et al., 2013). Indeed, distinct changes in the urinary proteome of patients at an early stage of diabetes might distinguish subjects at risk for progressive GFR loss, and hence at high risk of ESRD or cardiovascular events, even when no obvious clinical signs of renal dysfunction are present. On the other hand, a throughout understanding of the effects of genetic variability on the individual response to RAAS inhibitors, as well as of the cross-talk between the RAAS and other pathophysiological pathways, including the Klotho/FGF23 system (de Borst et al., 2011; Zoja et al., 2012), that might actually blunt the renoprotective effects of RAAS blocking agents in the presence of high phosphorus load (Zoccali et al., 2011), will hopefully help to improve the management of DN. Until new diagnostic and therapeutic options will become available, general practitioners and specialists should take into account the individual characteristics of diabetic patients in order to tailor intervention to specific individual patient needs. For instance, young patients with type 1 or type 2 diabetes—a dramatically increasing population (Kaufman, 2002)—will benefit the most from early intensified intervention aimed at optimizing the control of all treatable risk factors to minimize the risk of chronic complications and long-term cardiovascular morbidity and mortality. These benefits will largely override the potential excess risk of hypoglycaemia. Quite different will be the case of a type 2 diabetic patient, aged 70 years or older, with advanced end-organ damage and evidence of high risk of coronary or cerebrovascular disease. In this setting, intensified metabolic control would offer no advantages in terms of long-term prevention of complications and would expose the patient to potentially catastrophic consequences of hypoglycaemic episodes (Gerstein et al., 2008). In those with concomitant renal vascular disease—who will be the vast majority—intensified blood pressure control and/or RAAS inhibitor therapy might even accelerate renal disease progression and expose the patient to the risk of life-threatening hypotension episodes, in particular during intercurrent diseases or dehydration, an event not uncommon in the elderly (Law et al., 2009; Reboldi et al., 2010). Compliance and treatment-related hyperkalaemia may also be a concern, in particular in those on dual RAAS blockade. Actually, no evidence is available so far that combined ACEI and ARB therapy may offer any benefit in patients with type 2 diabetes with overt nephropathy, whereas this approach might reasonably provide major renoprotection to younger patients with less advanced renal disease. A response-driven approach, titrated to both efficacy and tolerability, and combined with close monitoring and patient counselling, will be the key component of effective interventions to minimize harm, particularly in in the most frail patients.

References

Abaterusso, C., Pertica, N., Lupo, A., *et al.* (2008). Anaemia in diabetic renal failure: is there a role for early erythropoietin treatment in preventing cardiovascular mortality? *Diabetes Obes Metab*, 10(10), 843–9.

Abbate, M., Cravedi, P., Iliev, I., *et al.* (2011). Prevention and treatment of diabetic retinopathy: evidence from clinical trials and perspectives. *Curr Diabetes Rev*, 7(3), 190–200.

Addis, T. (1948). *Glomerular Nephritis: Diagnosis and Treatment*. New York: Macmillan.

Adetunji, O. R., Mani, H., Olujohungbe, A., *et al.* (2009). 'Microalbuminuric anaemia'—the relationship between haemoglobin levels and albuminuria in diabetes. *Diabetes Res Clin Pract*, 85(2), 179–82.

Adler, A. I., Stevens, R. J., Manley, S. E., *et al.* (2003). Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney Int*, 63(1), 225–32.

Adler, A. I., Stratton, I. M., Neil, H. A., *et al.* (2000). Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36), prospective observational study. *BMJ*, 321(7258), 412–19.

Agarwal, R., Debella, Y. T., Giduma, H. D., *et al.* (2012). Long-term retinal, renal and cardiovascular outcomes in diabetic chronic kidney disease without proteinuria. *Nephrol Dial Transplant*, 27(1), 310–17.

Agrawal, L., Azad, N., Emanuele, N. V., *et al.* (2011). Observation on renal outcomes in the Veterans Affairs Diabetes Trial. *Diabetes Care*, 34(9), 2090–4.

Al-Khoury, S., Afzali, B., Shah, N., *et al.* (2006). Anaemia in diabetic patients with chronic kidney disease--prevalence and predictors. *Diabetologia*, 49(6), 1183–9.

Almdal, T., Norgaard, K., Feldt-Rasmussen, B., *et al.* (1994). The predictive value of microalbuminuria in IDDM. A five-year follow-up study. *Diabetes Care*, 17(2), 120–5.

American Diabetes Association (2012a). Executive summary: standards of medical care in diabetes – 2012. *Diabetes Care*, 35 Suppl 1, S4–10.

American Diabetes Association (2012b). Standards of medical care in diabetes – 2012. *Diabetes Care*, 35 Suppl 1, S11–63.

Anderson, S. and Brenner, B. M. (1986). The role of intraglomerular pressure in the initiation and progression of renal disease. *J Hyperten Suppl*, 4(5), S236–8.

Anderson, S. and Vora, J. P. (1995). Current concepts of renal hemodynamics in diabetes. *J Diabetes Complications*, 9(4), 304–7.

Ansquer, J. C., Foucher, C., Rattier, S., *et al.* (2005). Fenofibrate reduces progression to microalbuminuria over 3 years in a placebo-controlled study in type 2 diabetes: results from the Diabetes Atherosclerosis Intervention Study (DAIS). *Am J Kidney Dis*, 45(3), 485–93.

Arima, S. and Ito, S. (2003). The mechanisms underlying altered vascular resistance of glomerular afferent and efferent arterioles in diabetic nephropathy. *Nephrol Dial Transplant*, 18(10), 1966–9.

Arnlov, J., Evans, J. C., Meigs, J. B., *et al.* (2005). Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: the Framingham Heart Study. *Circulation*, 112(7), 969–75.

Astor, B. C., Muntner, P., Levin, A., *et al.* (2002). Association of kidney function with anemia: the Third National Health and Nutrition Examination Survey (1988–1994). *Arch Intern Med*, 162(12), 1401–8.

Astrup, A. S., Tarnow, L., Rossing, P., *et al.* (2005). Improved prognosis in type 1 diabetic patients with nephropathy: a prospective follow-up study. *Kidney Int*, 68(3), 1250–7.

Avram, M. M., Mittman, N., Myint, M. M., *et al.* (2001). Importance of low serum intact parathyroid hormone as a predictor of mortality in hemodialysis and peritoneal dialysis patients: 14 years of prospective observation. *Am J Kidney Dis*, 38(6), 1351–7.

Azoulay, L., Schneider-Lindner, V., Dell'aniello, S., *et al.* (2010). Thiazolidinediones and the risk of incident strokes in patients with type 2 diabetes: a nested case-control study. *Pharmacoepidemiol Drug Saf*, 19(4), 343–50.

Baba, S. (2001). Nifedipine and enalapril equally reduce the progression of nephropathy in hypertensive type 2 diabetics. *Diabetes Res Clin Pract*, 54(3), 191–201.

Bakris, G., Burgess, E., Weir, M., *et al.* (2008a). Telmisartan is more effective than losartan in reducing proteinuria in patients with diabetic nephropathy. *Kidney Int*, 74(3), 364–9.

Bakris, G. L., Barnhill, B. W., and Sadler, R. (1992). Treatment of arterial hypertension in diabetic humans: importance of therapeutic selection. *Kidney Int*, 41(4), 912–19.

Bakris, G. L., Copley, J. B., Vicknair, N., *et al.* (1996). Calcium channel blockers versus other antihypertensive therapies on progression of NIDDM associated nephropathy. *Kidney Int*, 50(5), 1641–50.

Bakris, G. L., Mangrum, A., Copley, J. B., *et al.* (1997). Effect of calcium channel or beta-blockade on the progression of diabetic nephropathy in African Americans. *Hypertension*, 29(3), 744–50.

Bakris, G. L., Ruilope, L. M., McMorn, S. O., *et al.* (2006). Rosiglitazone reduces microalbuminuria and blood pressure independently of glycemia in type 2 diabetes patients with microalbuminuria. *J Hyperten*, 24(10), 2047–55.

Bakris, G. L., Sarafidis, P. A., Weir, M. R., *et al.* (2010). Renal outcomes with different fixed-dose combination therapies in patients with hypertension at high risk for cardiovascular events (ACCOMPLISH): a prespecified secondary analysis of a randomised controlled trial. *Lancet*, 375(9721), 1173–81.

Bakris, G. L., Toto, R. D., McCullough, P. A., *et al.* (2008b). Effects of different ACE inhibitor combinations on albuminuria: results of the GUARD study. *Kidney Int*, 73(11), 1303–9.

Bakris, G. L., Weir, M. R., DeQuattro, V., *et al.* (1998). Effects of an ACE inhibitor/calcium antagonist combination on proteinuria in diabetic nephropathy. *Kidney Int*, 54(4), 1283–9.

Bakris, G. L., Weir, M. R., Secic, M., *et al.* (2004). Differential effects of calcium antagonist subclasses on markers of nephropathy progression. *Kidney Int*, 65(6), 1991–2002.

Bakris, G. L., Weir, M. R., Shanifar, S., *et al.* (2003). Effects of blood pressure level on progression of diabetic nephropathy: results from the RENAAL study. *Arch Intern Med*, 163(13), 1555–65.

Bangstad, H. J., Osterby, R., Dahl-Jorgensen, K., *et al.* (1994). Improvement of blood glucose control in IDDM patients retards the progression of morphological changes in early diabetic nephropathy. *Diabetologia*, 37(5), 483–90.

Barnett, A. H., Bain, S. C., Bouter, P., *et al.* (2004). Angiotensin-receptor blockade versus converting-enzyme inhibition in type 2 diabetes and nephropathy. *N Engl J Med*, 351(19), 1952–61.

Baron, A. D., Brechtel, G., Wallace, P., *et al.* (1998). Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *Am J Physiol*, 255(6 Pt 1), E769–74.

Barzilay, J., Warram, J. H., Bak, M., *et al.* (1992). Predisposition to hypertension: risk factor for nephropathy and hypertension in IDDM. *Kidney Int*, 41(4), 723–30.

Becker, G. J., Hewitson, T. D., and Chrysostomou, A. (2009). Aldosterone in clinical nephrology--old hormone, new questions. *Nephrol Dial Transplant*, 24(8), 2316–21.

Beck-Nielsen, H., Olesen, T., Mogensen, C. E., *et al.* (1990). Effect of near normoglycemia for 5 years on progression of early diabetic retinopathy and renal involvement. *Diabetes Res*, 15(4), 185–90.

Berl, T. and Henrich, W. (2006). Kidney-heart interactions: epidemiology, pathogenesis, and treatment. *ClinJ Am Soc Nephrol*, 1(1), 8–18.

Bianchi, S., Bigazzi, R., and Campese, V. M. (2006). Long-term effects of spironolactone on proteinuria and kidney function in patients with chronic kidney disease. *Kidney Int*, 70(12), 2116–23.

Bianchi, S., Bigazzi, R., and Campese, V. M. (1999). Microalbuminuria in essential hypertension: significance, pathophysiology, and therapeutic implications. *Am J Kidney Dis*, 34(6), 973–95.

Biesenbach, G., Grafinger, P., Janko, O., *et al.* (1997). Influence of cigarette-smoking on the progression of clinical diabetic nephropathy in type 2 diabetic patients. *Clin Nephrol*, 48(3), 146–50.

Bilous, R., Chaturvedi, N., Sjolie, A. K., *et al.* (2009). Effect of candesartan on microalbuminuria and albumin excretion rate in diabetes: three randomized trials. *Ann Intern Med*, 151(1), 11–14.

Bjorck, S., Mulec, H., Johnsen, S. A., *et al.* (1992). Renal protective effect of enalapril in diabetic nephropathy. *BMJ*, 304, 339–43.

Bjorn, S. F., Bangstad, H. J., Hanssen, K. F., *et al.* (1995). Glomerular epithelial foot processes and filtration slits in IDDM patients. *Diabetologia*, 38(10), 1197–204.

Bohle, A., Wehrmann, M., Bogenschutz, O., *et al.* (1991). The pathogenesis of chronic renal failure in diabetic nephropathy. Investigation of 488 cases of diabetic glomerulosclerosis. *Pathol Res Pract*, 187(2–3), 251–9.

Bomback, A. S. and Klemmer, P. J. (2007). The incidence and implications of aldosterone breakthrough. *Nat Clin Pract Nephrol*, 3(9), 486–92.

Borch-Johnsen, K. and Kreiner, S. (1987). Proteinuria: value as predictor of cardiovascular mortality in insulin dependent diabetes mellitus. *Br Med J (Clin Res Ed)*, 294(6588), 1651–4.

Borch-Johnsen, K., Norgaard, K., Hommel, E., *et al.* (1992). Is diabetic nephropathy an inherited complication? *Kidney Int*, 41(4), 719–22.

Braun, J., Oldendorf, M., Moshage, W., *et al.* (1996). Electron beam computed tomography in the evaluation of cardiac calcification in chronic dialysis patients. *Am J Kidney Dis*, 27(3), 394–401.

Brenner, B. M., Cooper, M. E., de Zeeuw, D., *et al.* (2001). Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med*, 345(12), 861–9.

Brodsky, I. G., Robbins, D. C., Hiser, E., *et al.* (1992). Effects of low-protein diets on protein metabolism in insulin-dependent diabetes mellitus patients with early nephropathy. *J Clin Endocrinol Metab*, 75(2), 351–7.

Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature*, 414(6865), 813–20.

Brownlee, M. (2005). The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*, 54(6), 1615–25.

Burns, W. C., Twigg, S. M., Forbes, J. M., *et al.* (2006). Connective tissue growth factor plays an important role in advanced glycation end product-induced tubular epithelial-to-mesenchymal transition: implications for diabetic renal disease. *J Am Soc Nephrol*, 17(9), 2484–94.

Burrows, N. R., Li, Y., and Geiss, L. S. (2010). Incidence of treatment for end-stage renal disease among individuals with diabetes in the U.S. continues to decline. *Diabetes Care*, 33(1), 73–7.

Buter, H., Hemmelder, M. H., Navis, G., *et al.* (1998). The blunting of the antiproteinuric efficacy of ACE inhibition by high sodium intake can be restored by hydrochlorothiazide. *Nephrol Dial Transplant*, 13(7), 1682–5.

Cabre, A., Lazaro, I., Girona, J., *et al.* (2008). Plasma fatty acid-binding protein 4 increases with renal dysfunction in type 2 diabetic patients without microalbuminuria. *Clin Chem*, 54(1), 181–7.

Callaghan, B. C., Little, A. A., Feldman, E. L., *et al.* (2012). Enhanced glucose control for preventing and treating diabetic neuropathy. *Cochrane Database Syst Rev*, 6, CD007543.

Cameron, J. S. (2003). Milk or albumin? The history of proteinuria before Richard Bright. *Nephrol Dial Transplant*, 18(7), 1281–5.

Campbell, R., Sangalli, F., Perticucci, E., *et al.* (2003). Effects of combined ACE inhibitor and angiotensin II antagonist treatment in human chronic nephropathies. *Kidney Int*, 63(3), 1094–103.

Caramori, M. L., Fioretto, P., and Mauer, M. (2003). Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients: an indicator of more advanced glomerular lesions. *Diabetes*, 52(4), 1036–40.

Casas, J. P., Chua, W., Loukogeorgakis, S., *et al.* (2005). Effect of inhibitors of the renin-angiotensin system and other antihypertensive drugs on renal outcomes: systematic review and meta-analysis. *Lancet*, 366(9502), 2026–33.

Cha, D. R., Zhang, X., Zhang, Y., *et al.* (2007). Peroxisome proliferator activated receptor alpha/gamma dual agonist tesaglitazar attenuates diabetic nephropathy in db/db mice. *Diabetes*, 56(8), 2036–45.

Chagnac, A., Weinstein, T., Herman, M., *et al.* (2003). The effects of weight loss on renal function in patients with severe obesity. *J Am Soc Nephrol*, 14(6), 1480–6.

Chaiken, R. L., Eckert-Norton, M., Bard, M., *et al.* (1998). Hyperfiltration in African-American patients with type 2 diabetes. Cross-sectional and longitudinal data. *Diabetes Care*, 21(12), 2129–34.

Chan, J. C., Cockram, C. S., Nicholls, M. G., *et al.* (1992). Comparison of enalapril and nifedipine in treating non-insulin dependent diabetes associated with hypertension: one year analysis. *BMJ*, 305(6860), 981–5.

Chaturvedi, N., Bandinelli, S., Mangili, R., *et al.* (2001). Microalbuminuria in type 1 diabetes: rates, risk factors and glycemic threshold. *Kidney Int*, 60(1), 219–27.

Chaturvedi, N., Porta, M., Klein, R., *et al.* (2008). Effect of candesartan on prevention (DIRECT-Prevent 1) and progression (DIRECT-Protect 1) of retinopathy in type 1 diabetes: randomised, placebo-controlled trials. *Lancet*, 372(9647), 1394–402.

Chen, S., Lee, J. S., Iglesias-De La Cruz, M. C., *et al.* (2005). Angiotensin II stimulates alpha3(IV) collagen production in mouse podocytes via TGF-beta and VEGF signalling: implications for diabetic glomerulopathy. *Nephrol Dial Transplant*, 20(7), 1320–8.

Cheng, A. and Frishman, W. H. (1998). Use of angiotensin-converting enzyme inhibitors as monotherapy and in combination with diuretics and calcium channel blockers. *J Clin Pharmacol*, 38(6), 477–91.

Chrysostomou, A. and Becker, G. (2001). Spironolactone in addition to ACE inhibition to reduce proteinuria in patients with chronic renal disease. *N Engl J Med*, 345(12), 925–6.

Cignarelli, M., Lamacchia, O., Di Paolo, S., *et al.* (2008). Cigarette smoking and kidney dysfunction in diabetes mellitus. *J Nephrol*, 21(2), 180–9.

Coca, S. G., Ismail-Beigi, F., Haq, N., *et al.* (2012). Role of intensive glucose control in development of renal end points in type 2 diabetes mellitus: systematic review and meta-analysis intensive glucose control in type 2 diabetes. *Arch Intern Med*, 172(10), 761–9.

Coimbra, T. M., Janssen, U., Grone, H. J., *et al.* (2000). Early events leading to renal injury in obese Zucker (fatty) rats with type II diabetes. *Kidney Int*, 57(1), 167–82.

Colberg, S. R., Swain, D. P., and Vinik, A. I. (2003). Use of heart rate reserve and rating of perceived exertion to prescribe exercise intensity in diabetic autonomic neuropathy. *Diabetes Care*, 26(4), 986–90.

Cooper, M. E., Vranes, D., Youssef, S., *et al.* (1999). Increased renal expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 in experimental diabetes. *Diabetes*, 48(11), 2229–39.

Cooper, M. E. (2001). Interaction of metabolic and haemodynamic factors in mediating experimental diabetic nephropathy. *Diabetologia*, 44(11), 1957–72.

Cooper-DeHoff, R. M., Gong, Y., Handberg, E. M., *et al.* (2010). Tight blood pressure control and cardiovascular outcomes among hypertensive patients with diabetes and coronary artery disease. *JAMA*, 304(1), 61–8.

Craig, K. J., Williams, J. D., Riley, S. G., *et al.* (2005). Anemia and diabetes in the absence of nephropathy. *Diabetes Care*, 28(5), 1118–23.

Cravedi, P., Ruggenenti, P., and Remuzzi, G. (2012). Proteinuria should be used as a surrogate in CKD. *Nat Rev Nephrol*, 8(5), 301–6.

Cravedi, P., Ruggenenti, P., and Remuzzi, G. (2010). Which antihypertensive drugs are the most nephroprotective and why? *Expert Opin Pharmacother*, 11, 2651–63.

Crepaldi, G., Carta, Q., Deferrari, G., *et al.* (1998). Effects of lisinopril and nifedipine on the progression to overt albuminuria in IDDM patients with incipient nephropathy and normal blood pressure. The Italian Microalbuminuria Study Group in IDDM. *Diabetes Care*, 21(1), 104–10.

Crook, E. D. and Patel, S. R. (2004). Diabetic nephropathy in African-American patients. *CurrDiabRep* 4(6), 455–61.

Dahl-Jorgensen, K., Bjoro, T., Kierulf, P., *et al.* (1992). Long-term glycemic control and kidney function in insulin-dependent diabetes mellitus. *Kidney Int*, 41(4), 920–3.

Dalla Vestra, M., Pozza, G., Mosca, A., *et al.* (2004). Effect of lercanidipine compared with ramipril on albumin excretion rate in hypertensive type 2 diabetic patients with microalbuminuria: DIAL study (diabete, ipertensione, albuminuria, lercanidipina). *Diabetes Nutr Metab*, 17(5), 259–66.

Dalla Vestra, M., Saller, A., Bortoloso, E., *et al.* (2000). Structural involvement in type 1 and type 2 diabetic nephropathy. *Diabetes Metab*, 26 Suppl 4, 8–14.

Daniels, M. C., McClain, D. A., and Crook, E. D. (2000). Transcriptional regulation of transforming growth factor beta1 by glucose: investigation

into the role of the hexosamine biosynthesis pathway. *Am J Med Sci*, 319(3), 138–42.
Daull, P., Jeng, A. Y., and Battistini, B. (2007). Towards triple vasopeptidase inhibitors for the treatment of cardiovascular diseases. *J Cardiovasc Pharmacol*, 50(3), 247–56.
Davis, B., Dei, C. A., Long, D. A., *et al.* (2007). Podocyte-specific expression of angiopoietin-2 causes proteinuria and apoptosis of glomerular endothelia. *J Am Soc Nephrol*, 18(8), 2320–9.
De Boer, I. H., Rue, T. C., Cleary, P. A., *et al.* (2011a). Long-term renal outcomes of patients with type 1 diabetes mellitus and microalbuminuria: an analysis of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications cohort. *Arch Intern Med*, 171(5), 412–20.
De Boer, I. H., Sun, W., Cleary, P. A., *et al.* (2011b). Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. *N Engl J Med*, 365(25), 2366–76.
De Boer, R. A., Azizi, M., Danser, A. J., *et al.* (2012). Dual RAAS suppression: recent developments and implications in light of the ALTITUDE study. *J Renin Angiotensin Aldosterone Syst*, 13(3), 409–12.
De Borst, M. H., Vervloet, M. G., ter Wee, P. M., *et al.* (2011). Cross talk between the renin-angiotensin-aldosterone system and vitamin D-FGF-23-klotho in chronic kidney disease. *J Am Soc Nephrol*, 22(9), 1603–9.
De Cosmo, S., Motterlini, N., Prudente, S., *et al.* (2009). Impact of the PPAR-gamma2 Pro12Ala polymorphism and ACE inhibitor therapy on new-onset microalbuminuria in type 2 diabetes: evidence from BENEDICT. *Diabetes*, 58(12), 2920–9.
De Vriese, A. S., Stoenoiu, M. S., Elger, M., *et al.* (2001a). Diabetes-induced microvascular dysfunction in the hydronephrotic kidney: role of nitric oxide. *Kidney Int*, 60(1), 202–10.
De Vriese, A. S., Tilton, R. G., Elger, M., *et al.* (2001b). Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. *J Am Soc Nephrol*, 12(5), 993–1000.
De Zeeuw, D. (2004). Albuminuria, not only a cardiovascular/renal risk marker, but also a target for treatment? *Kidney Int Suppl*, 92, S2–6.
De Zeeuw, D., Remuzzi, G., Parving, H. H., *et al.* (2004). Albuminuria, a therapeutic target for cardiovascular protection in type 2 diabetic patients with nephropathy. *Circulation*, 110(8), 921–7.
De Zeeuw, D., Akizawa, T., Audhya, P., *et al.* (2013). Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. *N Engl J Med*, 369(26), 2492–503.
Deckert, T., Feldt-Rasmussen, B., Borch-Johnsen, K., *et al.* (1989). Albuminuria reflects widespread vascular damage. The Steno hypothesis. *Diabetologia*, 32(4), 219–26.
Deckert, T., Kofoed-Enevoldsen, A., Norgaard, K., *et al.* (1992). Microalbuminuria. Implications for micro- and macrovascular disease. *Diabetes Care*, 15(9), 1181–91.
Dessapt, C., Karalliedde, J., Hernandez-Fuentes, M., *et al.* (2010). Circulating vascular progenitor cells in patients with type 1 diabetes and microalbuminuria. *Diabetes Care*, 33(4), 875–7.
Dessapt-Baradez, C., Woolf, A. S., White, K. E., *et al.* (2014). Targeted glomerular angiopoietin-1 therapy for early diabetic kidney disease. *J Am Soc Nephrol*, 25(1), 33–42.
Dhaun, N., Goddard, J., Kohan, D. E., *et al.* (2008). Role of endothelin-1 in clinical hypertension: 20 years on. *Hypertension*, 52(3), 452–9.
Di Carli, M. F., Bianco-Batlles, D., Landa, M. E., *et al.* (1999). Effects of autonomic neuropathy on coronary blood flow in patients with diabetes mellitus. *Circulation*, 100(8), 813–9.
Dluhy, R. G. and McMahon, G. T. (2008). Intensive glycemic control in the ACCORD and ADVANCE trials. *N Engl J Med*, 358(24), 2630–3.
Draznin, B., Sussman, K. E., Eckel, R. H., *et al.* (1988). Possible role of cytosolic free calcium concentrations in mediating insulin resistance of obesity and hyperinsulinemia. *J Clin Invest*, 82(6), 1848–52.
Du, J., Fan, Y. Y., Hitomi, H., *et al.* (2009). Mineralocorticoid receptor blockade and calcium channel blockade have different renoprotective effects on glomerular and interstitial injury in rats. *Am J Physiol Renal Physiol*, 297(3), F802–8.
Duckworth, W., Abraira, C., Moritz, T., *et al.* (2009). Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med*, 360(2), 129–39.
Dworkin, L. D., Hostetter, T. H., Rennke, H. G., *et al.* (1984). Hemodynamic basis for glomerular injury in rats with desoxycorticosterone-salt hypertension. *J Clin Invest*, 73(5), 1448–61.
Earle, K. A., Walker, J., Hill, C., *et al.* (1992). Familial clustering of cardiovascular disease in patients with insulin-dependent diabetes and nephropathy. *N Engl J Med*, 326, 673–7.
Eder, J., Hommel, U., Cumin, F., *et al.* (2007). Aspartic proteases in drug discovery. *Curr Pharm Des*, 13(3), 271–85.
EDIC Study Group (2000). Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. *N Engl J Med*, 342(6), 381–9.
Eknoyan, G., Hostetter, T., Bakris, G. L., *et al.* (2003). Proteinuria and other markers of chronic kidney disease: a position statement of the national kidney foundation (NKF) and the national institute of diabetes and digestive and kidney diseases (NIDDK). *Am J Kidney Dis*, 42(4), 617–22.
Epstein, M. (2009). Re-examining RAS-blocking treatment regimens for abrogating progression of chronic kidney disease. *Nat Clin Pract Nephrol*, 5(1), 12–13.
Epstein, M. and Bakris, G. (1996). Newer approaches to antihypertensive therapy. Use of fixed-dose combination therapy. *Arch Intern Med*, 156(17), 1969–78.
Epstein, M., Williams, G. H., Weinberger, M., *et al.* (2006). Selective aldosterone blockade with eplerenone reduces albuminuria in patients with type 2 diabetes. *Clin J Am Soc Nephrol*, 1(5), 940–51.
Estacio, R. O., Jeffers, B. W., Gifford, N., *et al.* (2000). Effect of blood pressure control on diabetic microvascular complications in patients with hypertension and type 2 diabetes. *Diabetes Care*, 23 Suppl 2, B54–64.
Estacio, R. O. (2009). Renin-angiotensin-aldosterone system blockade in diabetes: role of direct renin inhibitors. *Postgrad Med*, 121(3), 33–44.
EUCLID Study Group (1997). Randomised placebo-controlled trial of lisinopril in normotensive patients with insulin-dependent diabetes and normoalbuminuria or microalbuminuria. The EUCLID Study Group. *Lancet*, 349(9068), 1787–92.
Fadini, G. P., Sartore, S., Albiero, M., *et al.* (2006). Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. *Arterioscler Thromb Vasc Biol*, 26(9), 2140–6.
Fagerudd, J. A., Tarnow, L., Jacobsen, P., *et al.* (1998). Predisposition to essential hypertension and development of diabetic nephropathy in IDDM patients. *Diabetes*, 47(3), 439–44.
Faul, C., Asanuma, K., Yanagida-Asanuma, E., *et al.* (2007). Actin up: regulation of podocyte structure and function by components of the actin cytoskeleton. *Trends Cell Biol*, 17(9), 428–37.
Feldt-Rasmussen, B., Mathiesen, E. R., and Deckert, T. (1986). Effect of two years of strict metabolic control on progression of incipient nephropathy in insulin-dependent diabetes. *Lancet*, 2(8519), 1300–4.
Ferder, L., Daccordi, H., Martello, M., *et al.* (1992). Angiotensin converting enzyme inhibitors versus calcium antagonists in the treatment of diabetic hypertensive patients. *Hypertension*, 19(2 Suppl), II237–42.
Fernando, D. J., Hutchison, A., Veves, A., *et al.* (1991). Risk factors for non-ischaemic foot ulceration in diabetic nephropathy. *Diabet Med*, 8(3), 223–5.
Ferrier, C., Ferrari, P., Weidmann, P., *et al.* (1991). Antihypertensive therapy with Ca2+. Antagonist verapamil and/or ACE inhibitor enalapril in NIDDM patients. *Diabetes Care*, 14(10), 911–4.
Fiaschi, E., Andres, G., Giacomelli, F., *et al.* (1959). Renal histopathology in the para-nephritic nephrotic syndrome; optical and electron microscopic studies of kidney biopsies. *Scientia medica italica (English ed)*, 7(4), 639–742.
Fiaschi, E., Grassi, B., and Andres, G. (1952). [Renal function in diabetes mellitus]. *Rassegna di fisiopatologia clinica e terapeutica*, 24(4), 373–410.
Fioretto, P., Steffes, M. W., Sutherland, D. E., *et al.* (1995). Sequential renal biopsies in insulin-dependent diabetic patients: structural factors associated with clinical progression. *Kidney Int*, 48(6), 1929–35.

Flyvbjerg, A., Dagnaes-Hansen, F., De Vriese, A. S., *et al.* (2002). Amelioration of long-term renal changes in obese type 2 diabetic mice by a neutralizing vascular endothelial growth factor antibody. *Diabetes*, 51(10), 3090–4.

Fogari, R., Corradi, L., Zoppi, A., *et al.* (2007). Addition of manidipine improves the antiproteinuric effect of candesartan in hypertensive patients with type II diabetes and microalbuminuria. *Am J Hypentens*, 20(10), 1092–6.

Fogari, R., Derosa, G., Zoppi, A., *et al.* (2007). Effect of telmisartan-amlodipine combination at different doses on urinary albumin excretion in hypertensive diabetic patients with microalbuminuria. *Am J Hypentens*, 20(4), 417–22.

Fogari, R., Preti, P., Zoppi, A., *et al.* (2002). Effects of amlodipine fosinopril combination on microalbuminuria in hypertensive type 2 diabetic patients. *Am J Hypentens*, 15(12), 1042–9.

Food and Drug Administration (2010). *Pioglitazone and Rosiglitazone Cardiovascular Safety Meta-Analyses*. [Online] <http://wwwfdagov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/UCM224739pdf>

Food and Drug Administration (2011). *FDA Drug Safety Communication: Safety Review Update of Benicar (olmesartan) and Cardiovascular Events*. [Online] <http://wwwfdagov/Drugs/DrugSafety/ucm251268htm>

Forsblom, C., Harjutsalo, V., Thorn, L. M., *et al.* (2011). Competing-risk analysis of ESRD and death among patients with type 1 diabetes and macroalbuminuria. *J Am Soc Nephrol*, 22(3), 537–44.

Forsblom, C., Hiukka, A., Leinonen, E. S., *et al.* (2010). Effects of long-term fenofibrate treatment on markers of renal function in type 2 diabetes: the FIELD Helsinki substudy. *Diabetes Care*, 33(2), 215–20.

Forsblom, C. M., Groop, P. H., Ekstrand, A., *et al.* (1998). Predictors of progression from normoalbuminuria to microalbuminuria in NIDDM. *Diabetes Care*, 21(11), 1932–8.

Foster, A. V., Snowden, S., Grenfell, A., *et al.* (1995). Reduction of gangrene and amputations in diabetic renal transplant patients: the role of a special foot clinic. *Diabet Med*, 12(7), 632–5.

Frank, J. (2008). Managing hypertension using combination therapy. *Am Fam Physician*, 77(9), 1279–86.

Freedman, B. I., Bostrom, M., Daeihagh, P., *et al.* (2007). Genetic factors in diabetic nephropathy. *Clin J Am Soc Nephrol*, 2(6), 1306–16.

Freedman, B. I., Bowden, D. W., Rich, S. S., *et al.* (2008). Genome-wide linkage scans for renal function and albuminuria in type 2 diabetes mellitus: the Diabetes Heart Study. *Diabet Med* 25(3), 268–76.

Fried, L. F., Orchard, T. J., and Kasiske, B. L. (2001). Effect of lipid reduction on the progression of renal disease: a meta-analysis. *Kidney Int*, 59(1), 260–9.

Fried, L. F., Emanuele, N., Zhang, J. H., *et al.* (2013). Combined angiotensin inhibition for the treatment of diabetic nephropathy. *New Engl J Med*, 369, 1892–903.

Friedman, R., De Azevedo, M. J., and Gross, J. L. (1988). Is endogenous creatinine clearance still a reliable index of glomerular filtration rate in diabetic patients? *Braz J Med Biol Res*, 21(5), 941–4.

Fries, J. W., Sandstrom, D. J., Meyer, T. W., *et al.* (1989). Glomerular hypertrophy and epithelial cell injury modulate progressive glomerulosclerosis in the rat. *Lab Invest*, 60(2), 205–18.

Fruhbeck, G., Jebb, S. A., and Prentice, A. M. (1998). Leptin: physiology and pathophysiology. *ClinPhysiol*, 18(5), 399–419.

Fu, B. M. and Shen, S. (2003). Structural mechanisms of acute VEGF effect on microvessel permeability. *Am J Physiol Heart Circ Physiol*, 284(6), H2124–35.

Fujita, T., Ando, K., Nishimura, H., *et al.* (2007). Antiproteinuric effect of the calcium channel blocker cilnidipine added to renin-angiotensin inhibition in hypertensive patients with chronic renal disease. *Kidney Int*, 72(12), 1543–9.

Gaede, P., Lund-Andersen, H., Parving, H. H., *et al.* (2008). Effect of a multifactorial intervention on mortality in type 2 diabetes. *N Engl J Med*, 358(6), 580–91.

Gaede, P., Tarnow, L., Vedel, P., *et al.* (2004). Remission to normoalbuminuria during multifactorial treatment preserves kidney function in patients with type 2 diabetes and microalbuminuria. *Nephrol Dial Transplant*, 19(11), 2784–8.

Gaede, P., Vedel, P., Larsen, N., *et al.* (2003). Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med*, 348(5), 383–93.

Gall, M. A., Hougaard, P., Borch-Johnsen, K., *et al.* (1997). Risk factors for development of incipient and overt diabetic nephropathy in patients with non-insulin dependent diabetes mellitus: prospective, observational study. *BMJ*, 314(7083), 783–8.

Gallagher, A. M., Smeeth, L., Seabroke, S., *et al.* (2011). Risk of death and cardiovascular outcomes with thiazolidinediones: a study with the general practice research database and secondary care data. *PloS One*, 6(12), e28157.

Galle, J., Schwedhelm, E., Pinnetti, S., *et al.* (2008). Antiproteinuric effects of angiotensin receptor blockers: telmisartan versus valsartan in hypertensive patients with type 2 diabetes mellitus and overt nephropathy. *Nephrol Dial Transplant*, 23(10), 3174–83.

Gal-Moscovici, A. and Popovtzer, M. M. (2002). Parathyroid hormone-independent osteoclastic resorptive bone disease: a new variant of adynamic bone disease in haemodialysis patients. *Nephrol Dial Transplant*, 17(4), 620–4.

Gambara, V., Mecca, G., Remuzzi, G., *et al.* (1993). Heterogeneous nature of renal lesions in type II diabetes. *J Am Soc Nephrol*, 3(8), 1458–66.

Gaspari, F., Perico, N., and Remuzzi, G. (1997). Measurement of glomerular filtration rate. *Kidney Int Suppl*, 63, S151–4.

Gaspari, F., Perico, N., Ruggenenti, P., *et al.* (1995). Plasma clearance of nonradioactive iohexol as a measure of glomerular filtration rate. *J Am Soc Nephrol*, 6(2), 257–63.

Gaspari, F., Ruggenenti, P., Porrini, P., *et al.* (2013). The GFR and GFR decline cannot be accurately estimated in type 2 diabetics. *Kidney Int*, 84(1), 164–73.

Gassler, N., Elger, M., Kranzlin, B., *et al.* (2001). Podocyte injury underlies the progression of focal segmental glomerulosclerosis in the fa/fa Zucker rat. *Kidney Int*, 60(1), 106–16.

Genuth, S. (2006). Insights from the diabetes control and complications trial/epidemiology of diabetes interventions and complications study on the use of intensive glycemic treatment to reduce the risk of complications of type 1 diabetes. *EndocrPract*, 12 Suppl 1, 34–41.

Gerstein, H. C., Mann, J. F., Yi, Q., *et al.* (2001). Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA*, 286(4), 421–6.

Gerstein, H. C., Miller, M. E., Byington, R. P., *et al.* (2008). Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med*, 358(24), 2545–59.

Ghani, A. A., Al Waheeb, S., Al Sahow, A., *et al.* (2009). Renal biopsy in patients with type 2 diabetes mellitus: indications and nature of the lesions. *Ann Saudi Med*, 29(6), 450–3.

Gibbs, C. R., Beevers, D. G., and Lip, G. Y. H. (1999). The management of hypertensive disease in Black patients. *QJM*, 92(4), 187–92.

Gilbert, R. E. and Cooper, M. E. (1999). The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury? *Kidney Int*, 56(5), 1627–37.

Ginevri, F., Piccotti, E., Alinovi, R., *et al.* (1993). Reversible tubular proteinuria precedes microalbuminuria and correlates with the metabolic status in diabetic children. *Pediatr Nephrol*, 7(1), 23–6.

Giordano, M., Manzella, D., Paolisso, G., *et al.* (2001). Differences in heart rate variability parameters during the post-dialytic period in type II diabetic and non-diabetic ESRD patients. *Nephrol Dial Transplant*, 16(3), 566–73.

Gnudi, L., Thomas, S. M., and Viberti, G. (2007). Mechanical forces in diabetic kidney disease: a trigger for impaired glucose metabolism. *J Am Soc Nephrol*, 18(8), 2226–32.

Gnudi, L., Viberti, G., Raij, L., *et al.* (2003). GLUT-1 overexpression: link between hemodynamic and metabolic factors in glomerular injury? *Hypertension*, 42(1), 19–24.

Griffin, K. A., Kramer, H., and Bidani, A. K. (2008). Adverse renal consequences of obesity. *Am J Physiol Renal Physiol*, 294(4), F685–96.

Griffin, K. A., Picken, M. M., Bakris, G. L., *et al.* (1999). Class differences in the effects of calcium channel blockers in the rat remnant kidney model. *Kidney Int*, 55(5), 1849–60.

Groop, L., Ekstrand, A., Forsblom, C., *et al.* (1993). Insulin resistance, hypertension and microalbuminuria in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia*, 36(7), 642–7.

Gruden, G., Zonca, S., Hayward, A., *et al.* (2000). Mechanical stretch-induced fibronectin and transforming growth factor-beta1 production in human mesangial cells is p38 mitogen- activated protein kinase-dependent. *Diabetes*, 49(4), 655–61.

Guan, Y., Hao, C., Cha, D. R., *et al.* (2005). Thiazolidinediones expand body fluid volume through PPARgamma stimulation of ENaC-mediated renal salt absorption. *Nat Med*, 11(8), 861–6.

Guh, J. Y., Chen, H. C., Chuang, H. Y., *et al.* (2002). Risk factors and risk for mortality of mild hypoparathyroidism in hemodialysis patients. *Am J Kidney Dis*, 39(6), 1245–54.

Hall, J. E. (2003). The kidney, hypertension, and obesity. *Hypertension*, 41(3 Pt 2), 625–33.

Hall, J. E., Henegar, J. R., Dwyer, T. M., *et al.* (2004). Is obesity a major cause of chronic kidney disease? *Adv Ren Replace Ther*, 11(1), 41–54.

Hall, J. E., Jones, D. W., Kuo, J. J., *et al.* (2003). Impact of the obesity epidemic on hypertension and renal disease. *Curr Hypertens Rep*, 5(5), 386–92.

Haller, H., Ito, S., Izzo, J. L., Jr., *et al.* (2011). Olmesartan for the delay or prevention of microalbuminuria in type 2 diabetes. *N Engl J Med*, 364(10), 907–17.

Hanahan, D. (1997). Signaling vascular morphogenesis and maintenance. *Science*, 277(5322), 48–50.

Haneda, M., Kikkawa, R., Togawa, M., *et al.* (1992). High blood pressure is a risk factor for the development of microalbuminuria in Japanese subjects with non-insulin-dependent diabetes mellitus. *J Diabetes Complications*, 6(3), 181–5.

Harada, K., Akai, Y., Sumida, K., *et al.* (2012). Significance of renal biopsy in patients with presumed diabetic nephropathy. *J Diabet Invest*, 4, 88–93

Haraldsson, B., Nystrom, J., and Deen, W. M. (2008). Properties of the glomerular barrier and mechanisms of proteinuria. *Physiol Rev*, 88(2), 451–87.

Harel, Z., Gilbert, C., Wald, R., *et al.* (2012). The effect of combination treatment with aliskiren and blockers of the renin-angiotensin system on hyperkalaemia and acute kidney injury: systematic review and meta-analysis. *BMJ*, 344, e42.

Harris, D., Thomas, M., Johnson, D., *et al.* (2006). Caring for Australasians with Renal I. The CARI guidelines. Prevention of progression of kidney disease. *Nephrology*, 11 Suppl 1, S2–197.

Harris, R. C. (2013). The best laid plans. *Am J Physiol Renal Physiol*, 304(6), F808–19.

Hasslacher, C., Bostedt-Kiesel, A., Kempe, H. P., *et al.* (1993). Effect of metabolic factors and blood pressure on kidney function in proteinuric type 2 (non-insulin-dependent) diabetic patients. *Diabetologia*, 36(10), 1051–6.

Hasslacher, C., Stech, W., Wahl, P., *et al.* (1985). Blood pressure and metabolic control as risk factors for nephropathy in type 1 (insulin-dependent) diabetes. *Diabetologia*, 28(1), 6–11.

Hattori, S. (2011). Sitagliptin reduces albuminuria in patients with type 2 diabetes. *Endocrine J*, 58(1), 69–73.

Hayashi, K., Nagahama, T., Oka, K., *et al.* (1996). Disparate effects of calcium antagonists on renal microcirculation. *Hypertens Res*, 19(1), 31–6.

Hayashi, K., Ozawa, Y., Fujiwara, K., *et al.* (2003). Role of actions of calcium antagonists on efferent arterioles--with special references to glomerular hypertension. *Am J Nephrol*, 23(4), 229–44.

Hayashi, K., Wakino, S., Sugano, N., *et al.* (2007). Ca^{2+} channel subtypes and pharmacology in the kidney. *Circ Res*, 100(3), 342–53.

Heart Outcomes Prevention Evaluation (HOPE) Study Investigators (2000). Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. *Lancet*, 355, 253–9.

Heerspink, H. L. and de Zeeuw, D. (2010). Composite renal endpoints: was ACCOMPLISH accomplished? *Lancet*, 375(9721), 1140–2.

Heerspink, H. L., Greene, T., Lewis, J. B., *et al.* (2008). Effects of sulodexide in patients with type 2 diabetes and persistent albuminuria. *Nephrol Dial Transplant*, 23(6), 1946–54.

Heilig, C. W., Concepcion, L. A., Riser, B. L., *et al.* (1995). Overexpression of glucose transporters in rat mesangial cells cultured in a normal glucose milieu mimics the diabetic phenotype. *J Clin Invest*, 96(4), 1802–14.

Heilig, C. W., Kreisberg, J. I., Freytag, S., *et al.* (2001). Antisense GLUT-1 protects mesangial cells from glucose induction of GLUT-1 and fibronectin expression. *Am J Physiol Renal Physiol*, 280(4), F657–66.

Henegar, J. R., Bigler, S. A., Henegar, L. K., *et al.* (2001). Functional and structural changes in the kidney in the early stages of obesity. *J Am Soc Nephrol*, 12(6), 1211–17.

Hermans, M. M., Henry, R. M., Dekker, J. M., *et al.* (2008). Albuminuria, but not estimated glomerular filtration rate, is associated with maladaptive arterial remodeling: the Hoorn Study. *J Hyperten*, 26(4), 791–7.

Hillege, H. L., Fidler, V., Diercks, G. F., *et al.* (2002). Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation*, 106(14), 1777–82.

Hohenstein, B., Hausknecht, B., Boehmer, K., *et al.* (2006). Local VEGF activity but not VEGF expression is tightly regulated during diabetic nephropathy in man. *Kidney Int*, 69(9), 1654–61.

Hohenstein, B., Hugo, C. P., Hausknecht, B., *et al.* (2008). Analysis of NO-synthase expression and clinical risk factors in human diabetic nephropathy. *Nephrol Dial Transplant*, 23(4), 1346–54.

Hohenstein, B., Kuo, M. C., Addabbo, F., *et al.* (2010). Enhanced progenitor cell recruitment and endothelial repair after selective endothelial injury of the mouse kidney. *Am J Physiol Renal Physiol*, 298(6), F1504–14.

Holman, R. R., Paul, S. K., Bethel, M. A., *et al.* (2008a). 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med*, 359(15), 1577–89.

Holman, R. R., Paul, S. K., Bethel, M. A., *et al.* (2008b). Long-term follow-up after tight control of blood pressure in type 2 diabetes. *N Engl J Med*, 359(15), 1565–76.

Holtkamp, F. A., de Zeeuw, D., de Graeff, P. A., *et al.* (2011). Albuminuria and blood pressure, independent targets for cardioprotective therapy in patients with diabetes and nephropathy: a post hoc analysis of the combined RENAAL and IDNT trials. *Eur Heart J*, 32(12), 1493–9.

Hoshi, S., Shu, Y., Yoshida, F., *et al.* (2002). Podocyte injury promotes progressive nephropathy in zucker diabetic fatty rats. *Lab Invest*, 82(1), 25–35.

Hostetter, T. H. (1994). Mechanisms of diabetic nephropathy. *Am J Kidney Dis*, 23(2), 188–92.

Hostetter, T. H., Olson, J. L., Rennke, H. G., *et al.* (1981). Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *Am J Physiol*, 241(1), F85–93.

Hostetter, T. H., Rennke, H. G., and Brenner, B. M. (1982). The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. *Am J Med*, 72(3), 375–80.

Hostetter, T. H., Troy, J. L., and Brenner, B. M. (1981). Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int*, 19(3), 410–5.

Hovind, P., Rossing, P., Tarnow, L., *et al.* (2001). Progression of diabetic nephropathy. *Kidney Int*, 59(2), 702–9.

Hovind, P., Rossing, P., Tarnow, L., *et al.* (2001). Remission of nephrotic-range albuminuria in type 1 diabetic patients. *Diabetes Care*, 24(11), 1972–7.

Hovind, P., Tarnow, L., Rossing, P., *et al.* (2004). Predictors for the development of microalbuminuria and macroalbuminuria in patients with type 1 diabetes: inception cohort study. *BMJ*, 328(7448), 1105.

Huang, F., Yang, Q., Chen, L., *et al.* (2007). Renal pathological change in patients with type 2 diabetes is not always diabetic nephropathy: a report of 52 cases. *Clin Nephrol*, 67(5), 293–7.

Hull, R. L., Shen, Z. P., Watts, M. R., *et al.* (2005). Long-term treatment with rosiglitazone and metformin reduces the extent of, but does not prevent, islet amyloid deposition in mice expressing the gene for human islet amyloid polypeptide. *Diabetes*, 54(7), 2235–44.

Hummel, C. S., Lu, C., Loo, D. D., *et al.* (2011). Glucose transport by human renal Na+/D-glucose cotransporters SGLT1 and SGLT2. *Am J Physiol Cell Physiol*, 300(1), C14–21.

Ibsen, H., Olsen, M. H., Wachtell, K., *et al.* (2005). Reduction in albuminuria translates to reduction in cardiovascular events in hypertensive patients: losartan intervention for endpoint reduction in hypertension study. *Hypertension*, 45(2), 198–202.

Ibsen, H., Olsen, M. H., Wachtell, K., *et al.* (2006). Does albuminuria predict cardiovascular outcomes on treatment with losartan versus atenolol in patients with diabetes, hypertension, and left ventricular hypertrophy? The LIFE study. *Diabetes Care*, 29(3), 595–600.

Ichihara, A., Hayashi, M., Kaneshiro, Y., *et al.* (2004). Inhibition of diabetic nephropathy by a decoy peptide corresponding to the 'handle' region for nonproteolytic activation of prorenin. *J Clin Invest*, 114(8), 1128–35.

Ichinose, K., Maeshima, Y., Yamamoto, Y., *et al.* (2006). 2-(8-hydroxy-6-methoxy-1-oxo-1H-2-benzopyran-3-yl) propionic acid, an inhibitor of angiogenesis, ameliorates renal alterations in obese type 2 diabetic mice. *Diabetes*, 55(5), 1232–42.

Ichinose, K., Maeshima, Y., Yamamoto, Y., *et al.* (2005). Antiangiogenic endostatin peptide ameliorates renal alterations in the early stage of a type 1 diabetic nephropathy model. *Diabetes*, 54(10), 2891–903.

Igo, R. P., Jr., Iyengar, S. K., Nicholas, S. B., *et al.* (2011). Genomewide linkage scan for diabetic renal failure and albuminuria: the FIND study. *Am J Nephrol*, 33(5), 381–9.

Imai, E., Chan, J. C., Ito, S., *et al.* (2011). Effects of olmesartan on renal and cardiovascular outcomes in type 2 diabetes with overt nephropathy: a multicentre, randomised, placebo-controlled study. *Diabetologia*, 54(12), 2978–86.

Imano, E., Kanda, T., Nakatani, Y., *et al.* (1998). Effect of troglitazone on microalbuminuria in patients with incipient diabetic nephropathy. *Diabetes Care*, 21(12), 2135–9.

Inaba, M., Nagasue, K., Okuno, S., *et al.* (2002). Impaired secretion of parathyroid hormone, but not refractoriness of osteoblast, is a major mechanism of low bone turnover in hemodialyzed patients with diabetes mellitus. *Am J Kidney Dis*, 39(6), 1261–9.

Inaba, M., Okuno, S., Nagasue, K., *et al.* (2001). Impaired secretion of parathyroid hormone is coherent to diabetic hemodialyzed patients. *Am J Kidney Dis*, 38(4 Suppl 1), S139–42.

Inomata, S., Itoh, M., Imai, H., *et al.* (1997). Serum levels of erythropoietin as a novel marker reflecting the severity of diabetic nephropathy. *Nephron*, 75(4), 426–30.

Ismail-Beigi, F., Craven, T., Banerji, M. A., *et al.* (2010). Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. *Lancet*, 376(9739), 419–30.

Ito, S. and Abe, K. (1997). Contractile properties of afferent and efferent arterioles. *Clin Exp Pharmacol Physiol*, 24(7), 532–5.

Izzo, J. L., Jr. and Zion, A. S. (2011). Value of angiotensin receptor blocker therapy in diabetes. *J Clin Hypertens*, 13(4), 290–5.

Jacobsen, P., Andersen, S., Jensen, B. R., *et al.* (2003a). Additive effect of ACE inhibition and angiotensin II receptor blockade in type I diabetic patients with diabetic nephropathy. *J Am Soc Nephrol*, 14(4), 992–9.

Jacobsen, P., Andersen, S., Rossing, K., *et al.* (2003b). Dual blockade of the renin-angiotensin system versus maximal recommended dose of ACE inhibition in diabetic nephropathy. *Kidney Int*, 63(5), 1874–80.

Jacobsen, P., Andersen, S., Rossing, K., *et al.* (2002). Dual blockade of the renin-angiotensin system in type 1 patients with diabetic nephropathy. *Nephrol Dial Transplant*, 17(6), 1019–24.

Jamerson, K. A. (2005). Preventing chronic kidney disease in special populations. *Am J Hypertens*, 18(4 Pt 2), 106S–11S.

Jeansson, M., Gawlik, A., Anderson, G., *et al.* (2011). Angiopoietin-1 is essential in mouse vasculature during development and in response to injury. *J Clin Invest*, 121(6), 2278–89.

Jerums, G., Panagiotopoulos, S., Premaratne, E., *et al.* (2008). Lowering of proteinuria in response to antihypertensive therapy predicts improved renal function in late but not in early diabetic nephropathy: a pooled analysis. *Am J Nephrol*, 28(4), 614–27.

Jones, C. A., Krolewski, A. S., Rogus, J., *et al.* (2005). Epidemic of end-stage renal disease in people with diabetes in the United States population: do we know the cause? *Kidney Int*, 67(5), 1684–91.

Kagami, S., Border, W. A., Miller, D. E., *et al.* (1994). Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-beta expression in rat glomerular mesangial cells. *J Clin Invest*, 93(6), 2431–7.

Karalliedde, J. and Gnudi, L. (2011). Endothelial factors and diabetic nephropathy. *Diabetes Care*, 34 Suppl 2, S291–6.

Karalliedde, J. and Viberti, G. (2004). Microalbuminuria and cardiovascular risk. *Am J Hyperten*, 17(10), 986–93.

Karalliedde, J. and Viberti, G. (2010). Proteinuria in diabetes: bystander or pathway to cardiorenal disease? *J Am Soc Nephrol*, 21(12), 2020–7.

Kaufman, F. R. (2002). Type 2 diabetes mellitus in children and youth: a new epidemic. *J Pediatr Endocrinol Metab*, 15 Suppl 2, 737–44.

Keane, W. F. and Lyle, P. A. (2003). Recent advances in management of type 2 diabetes and nephropathy: lessons from the RENAAL study. *Am J Kidney Dis*, 41(3 Suppl 1), S22–5.

Keen, H., Chlouverakis, C., Fuller, J., *et al.* (1969). The consomitants of raised blood sugar: studies in newly-detected hyperglycaemics. II. Urinary albumin excretion, blood pressure and their relation to blood sugar levels. *Guys Hosp Rep*, 118(2), 247–54.

Khosla, N., Kalaitzidis, R., and Bakris, G. L. (2009). Predictors of hyperkalemia risk following hypertension control with aldosterone blockade. *Am J Nephrol*, 30(5), 418–24.

Kidney Disease Improving Global Outcomes (KDIGO) (2013). KDIGO 2012 Clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl*, 3(1), 1–150.

Kimmelstiel, P. and Wilson, C. (1936a). Benign and malignant hypertension and nephrosclerosis: a clinical and pathological study. *Am J Pathol*, 12(1), 45–82.3.

Kimmelstiel P. and Wilson C. (1936b). Intercapillary lesions in the glomeruli of the kidney. *Am J Pathol*, 12(1), 83–98.7.

Klahr, S., Levey, A. S., Beck, G. J., *et al.* (1994). The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group. *N Engl J Med*, 330(13), 877–84.

Klausen, K., Borch-Johnsen, K., Feldt-Rasmussen, B., *et al.* (2004). Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. *Circulation*, 110(1), 32–5.

Kohan, D. E., Pritchett, Y., Molitch, M., *et al.* (2011). Addition of atrasentan to renin-angiotensin system blockade reduces albuminuria in diabetic nephropathy. *J Am Soc Nephrol*, 22(4), 763–72.

Kohner, E. M. (2008). Microvascular disease: what does the UKPDS tell us about diabetic retinopathy? *Diabet Med*, 25 Suppl 2, 20–4.

Kramer, H. J., Nguyen, Q. D., Curhan, G., *et al.* (2003). Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. *JAMA*, 289(24), 3273–7.

Krishnan, A. V. and Kiernan, M. C. (2007). Uremic neuropathy: clinical features and new pathophysiological insights. *Muscle Nerve*, 35(3), 273–90.

Krishnan, A. V., Phoon, R. K., Pussell, B. A., *et al.* (2005). Altered motor nerve excitability in end-stage kidney disease. *Brain*, 128(Pt 9), 2164–74.

Kriz, W., Elger, M., Nagata, M., *et al.* (1994). The role of podocytes in the development of glomerular sclerosis. *Kidney Int Suppl*, 45, S64–72.

Krolewski, A S., Canessa, M., Warram, J. H., *et al.* (1988). Predisposition to hypertension and susceptibility to renal disease in insulin-dependent diabetes mellitus. *N Engl J Med*, 318(3), 140–5.

Ku, C. H., White, K. E., Dei, C. A., *et al.* (2008). Inducible overexpression of sFlt-1 in podocytes ameliorates glomerulopathy in diabetic mice. *Diabetes*, 57(10), 2824–33.

Kunz, R., Friedrich, C., Wolbers, M., *et al.* (2008). Meta-analysis: effect of monotherapy and combination therapy with inhibitors of the renin angiotensin system on proteinuria in renal disease. *Ann Intern Med*, 148(1), 30–48.

Lachin, J. M. (2010). Point: Intensive glycemic control and mortality in ACCORD—a chance finding? *Diabetes Care*, 33(12), 2719–21.

Laffel, L. M., McGill, J. B., and Gans, D. J. (1995). The beneficial effect of angiotensin-converting enzyme inhibition with captopril on diabetic nephropathy in normotensive IDDM patients with microalbuminuria. North American Microalbuminuria Study Group. *Am J Med*, 99(5), 497–504.

Lai, K. N., Chan, L. Y., Guo, H., *et al.* (2011). Additive effect of PPAR-gamma agonist and ARB in treatment of experimental IgA nephropathy. *Pediatr Nephrol*, 26(2), 257–66.

Lambers Heerspink, H. J., Gansevoort, R. T., Brenner, B. M., *et al.* (2010). Comparison of different measures of urinary protein excretion for prediction of renal events. *J Am Soc Nephrol*, 21(8), 1355–60.

Law, M. R., Morris, J. K., and Wald, N. J. (2009). Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. *BMJ*, 338, b1665.

Law, M. R., Wald, N. J., Morris, J. K., *et al.* (2003). Value of low dose combination treatment with blood pressure lowering drugs: analysis of 354 randomised trials. *BMJ*, 326(7404), 1427.

Lehrke, M. and Lazar, M. A. (2005). The many faces of PPARgamma. *Cell*, 123(6), 993–9.

Lemley, K. V., Blouch, K., Abdullah, I., *et al.* (2000). Glomerular permselectivity at the onset of nephropathy in type 2 diabetes mellitus. *J Am Soc Nephrol*, 11(11), 2095–105.

Levey, A. S., Coresh, J., Greene, T., *et al.* (2006). Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med*, 145(4), 247–54.

Levey, A. S., de Jong, P. E., Coresh, J., *et al.* (2011). The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int*, 80(1), 17–28.

Lewis, E. J., Greene, T., Spitalewiz, S., *et al.* (2012). Pyridorin in type 2 diabetic nephropathy. *J Am Soc Nephrol*, 23(1), 131–6.

Lewis, E. J., Hunsicker, L. G., Bain, R. P., *et al.* (1993). The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med*, 329(20), 1456–62.

Lewis, E. J., Hunsicker, L. G., Clarke, W. R., *et al.* (2001). Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med*, 345(12), 851–60.

Lewis, J. B., Berl, T., Bain, R. P., *et al.* (1999). Effect of intensive blood pressure control on the course of type 1 diabetic nephropathy. Collaborative Study Group. *Am J Kidney Dis*, 34(5), 809–17.

Li, Y., Wen, X., Spataro, B. C., *et al.* (2006). Hepatocyte growth factor is a downstream effector that mediates the antifibrotic action of peroxisome proliferator-activated receptor-gamma agonists. *J Am Soc Nephrol*, 17(1), 54–65.

Lim, H. S., Lip, G. Y., and Blann, A. D. (2005). Angiopoietin-1 and angiopoietin-2 in diabetes mellitus: relationship to VEGF, glycaemic control, endothelial damage/dysfunction and atherosclerosis. *Atherosclerosis*, 180(1), 113–18.

Lim, S. C., Caballero, A. E., Smakowski, P., *et al.* (1999). Soluble intercellular adhesion molecule, vascular cell adhesion molecule, and impaired microvascular reactivity are early markers of vasculopathy in type 2 diabetic individuals without microalbuminuria. *Diabetes Care*, 22(11), 1865–70.

Lindeman, R. D., Tobin, J., and Shock, N. W. (1985). Longitudinal studies on the rate of decline in renal function with age. *J Am Geriatr Soc*, 33(4), 278–85.

Liu, J. E., Robbins, D. C., Palmieri, V., *et al.* (2003). Association of albuminuria with systolic and diastolic left ventricular dysfunction in type 2 diabetes: the Strong Heart Study. *J Am Coll Cardiol*, 41(11), 2022–8.

Locatelli, F., Carbarns, I. R., Maschio, G., *et al.* (1997). Long-term progression of chronic renal insufficiency in the AIPRI Extension Study. The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study Group. *Kidney Int Suppl*, 63, S63–6.

London, G. M., Guerin, A. P., Marchais, S. J., *et al.* (2003). Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant*, 18(9), 1731–40.

London, G. M., Marty, C., Marchais, S. J., *et al.* (2004). Arterial calcifications and bone histomorphometry in end-stage renal disease. *J Am Soc Nephrol*, 15(7), 1943–51.

Lown, B. and Verrier, R. L. (1976). Neural activity and ventricular fibrillation. *N Engl J Med*, 294(21), 1165–70.

Lundbaek, K. (1970). [Renal structure and function in early and diabetes long-term]. *Rev Med Suisse Romande*, 90(8), 603–14.

Lurbe, E., Redon, J., Kesani, A., *et al.* (2002). Increase in nocturnal blood pressure and progression to microalbuminuria in type 1 diabetes. *N Engl J Med*, 347(11), 797–805.

Macdougall, I. C. and Cooper, A. C. (2002). Erythropoietin resistance: the role of inflammation and pro-inflammatory cytokines. *Nephrol Dial Transplant*, 17 Suppl 11, 39–43.

MacIsaac, R. J., Tsalamandris, C., Panagiotopoulos, S., *et al.* (2004). Nonalbuminuric renal insufficiency in type 2 diabetes. *Diabetes Care*, 27(1), 195–200.

Maddox, D. A. and Brenner, B. M. (2000). Glomerular ultrafiltration. In B. M. Brenner and F. C. Rector Jr. (eds.) *The Kidney*, pp. 319–74. Philadelphia, PA: W.B. Saunders Company.

Magee, G. M., Bilous, R. W., Cardwell, C. R., *et al.* (2009). Is hyperfiltration associated with the future risk of developing diabetic nephropathy? A meta-analysis. *Diabetologia*, 52(4), 691–7.

Maione, A., Navaneethan, S. D., Graziano, G., *et al.* (2011). Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and combined therapy in patients with micro- and macroalbuminuria and other cardiovascular risk factors: a systematic review of randomized controlled trials. *Nephrol Dial Transplant*, 26(9), 2827–47.

Makino, H., Haneda, M., Babazono, T., *et al.* (2007). Prevention of transition from incipient to overt nephropathy with telmisartan in patients with type 2 diabetes. *Diabetes Care*, 30(6), 1577–8.

Makino, H., Okada, S., Nagumo, A., *et al.* (2009). Decreased circulating CD34+ cells are associated with progression of diabetic nephropathy. *Diabet Med*, 26(2), 171–3.

Mallamaci, F., Tripepi, G., and Zoccali, C. (2005). Leptin in end stage renal disease (ESRD): a link between fat mass, bone and the cardiovascular system. *J Nephrol*, 18(4), 464–8.

Mann, J. F., Green, D., Jamerson, K., *et al.* (2010). Avosentan for overt diabetic nephropathy. *J Am Soc Nephrol*, 21(3), 527–35.

Mann, J. F., Schmieder, R. E., McQueen, M., *et al.* (2008). Renal outcomes with telmisartan, ramipril, or both, in people at high vascular risk (the ONTARGET study): a multicentre, randomised, double-blind, controlled trial. *Lancet*, 372(9638), 547–53.

Marsden, P A. (2009). Treatment of anemia in chronic kidney disease--strategies based on evidence. *N Engl J Med*, 361(21), 2089–90.

Martinez-Martin, F. J. and Saiz-Satjes, M. (2008). Add-on manidipine versus amlodipine in diabetic patients with hypertension and microalbuminuria: the AMANDHA study. *Expert Rev Cardiovasc Ther*, 6(10), 1347–55.

Maschio, G., Alberti, D., Janin, G., *et al.* (1996). Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study Group. *N Engl J Med*, 334(15), 939–45.

Massry, S. G. (1987). Parathyroid hormone: a uremic toxin. *Adv Exp Med Biol*, 223, 1–17.

Matsui, T., Yamagishi, S., Takeuchi, M., *et al.* (2010). Nifedipine inhibits advanced glycation end products (AGEs) and their receptor (RAGE) interaction-mediated proximal tubular cell injury via peroxisome proliferator-activated receptor-gamma activation. *Biochem Biophys Res Commun*, 398(2), 326–30.

Matsui, T., Yamagishi, S., Ueda, S., *et al.* (2007). Telmisartan, an angiotensin II type 1 receptor blocker, inhibits advanced glycation end-product (AGE)-induced monocyte chemoattractant protein-1 expression in mesangial cells through downregulation of receptor for AGEs via peroxisome proliferator-activated receptor-gamma activation. *J Int Med Res*, 35(4), 482–9.

Mauer, M., Zinman, B., Gardiner, R., *et al.* (2009). Renal and retinal effects of enalapril and losartan in type 1 diabetes. *N Engl J Med*, 361(1), 40–51.

Mauer, S. M., Steffes, M. W., Ellis, E. N., *et al.* (1984). Structural-functional relationships in diabetic nephropathy. *J Clin Invest*, 74(4), 1143–55.

McCormick, B. B., Sydor, A., Akbari, A., *et al.* (2008). The effect of pentoxifylline on proteinuria in diabetic kidney disease: a meta-analysis. *Am J Kidney Dis*, 52(3), 454–63.

McNair, P. (1988). Bone mineral metabolism in human type 1 (insulin dependent) diabetes mellitus. *Dan Med Bull*, 35(2), 109–21.

Mehdi, U. F., Adams-Huet, B., Raskin, P., *et al.* (2009). Addition of angiotensin receptor blockade or mineralocorticoid antagonism to maximal angiotensin-converting enzyme inhibition in diabetic nephropathy. *J Am Soc Nephrol*, 20(12), 2641–50.

Menne, J., Chatzikyrkou, C., and Haller, H. (2010). Microalbuminuria as a risk factor: the influence of renin-angiotensin system blockade. *J Hyperten*, 28(10), 1983–94.

Meyer, T. W., Bennett, P. H., and Nelson, R. G. (1999). Podocyte number predicts long term urinary albumin excretion in Pima Indians with type II diabetes and microalbuminuria. *Diabetologia*, 42, 1341–4.

Microalbuminuria Collaborative Study Group, United Kingdom (1995). Intensive therapy and progression to clinical albuminuria in patients with insulin dependent diabetes mellitus and microalbuminuria. *BMJ*, 311(7011), 973–7.

Miettinen, H., Haffner, S. M., Lehto, S., *et al.* (1996). Proteinuria predicts stroke and other atherosclerotic vascular disease events in nondiabetic and non-insulin-dependent diabetic subjects. *Stroke*, 27(11), 2033–9.

Mimran, A., Insua, A., Ribstein, J., *et al.* (1988). Comparative effect of captopril and nifedipine in normotensive patients with incipient diabetic nephropathy. *Diabetes Care*, 11(10), 850–3.

Miyazaki, Y., Cersosimo, E., Triplitt, C., *et al.* (2007). Rosiglitazone decreases albuminuria in type 2 diabetic patients. *Kidney Int*, 72(11), 1367–73.

Moffett, S. P., Zmuda, J. M., Cauley, J. A., *et al.*(2004). Association of the G-174C variant in the interleukin-6 promoter region with bone loss and fracture risk in older women. *J Bone Miner Res*, 19(10), 1612–18.

Mogensen, C. E. (1971). Glomerular filtration rate and renal plasma flow in short-term and long-term juvenile diabetes mellitus. *Scand J Clin Lab Investigat*, 28(1), 91–100.

Mogensen, C. E. (1986). Early glomerular hyperfiltration in insulin-dependent diabetics and late nephropathy. *Scand J Clin Lab Invest*, 46(3), 201–6.

Mogensen, C. E., Neldam, S., Tikkanen, I., *et al.* (2000). Randomised controlled trial of dual blockade of renin-angiotensin system in patients with hypertension, microalbuminuria, and non-insulin dependent diabetes: the candesartan and lisinopril microalbuminuria (CALM) study. *BMJ*, 321(7274), 1440–4.

Mogensen, C. E., Vestbo, E., Poulsen, P. L., *et al.* (1995). Microalbuminuria and potential confounders. A review and some observations on variability of urinary albumin excretion. *Diabetes Care*, 18(4), 572–81.

Mogensen, C. E., Viberti, G., Halimi, S., *et al.* (2003). Effect of low-dose perindopril/indapamide on albuminuria in diabetes: preterax in albuminuria regression: PREMIER. *Hypertension*, 41(5), 1063–71.

Molitch, M. E., DeFronzo, R. A., Franz, M. J., *et al.* (2004). Nephropathy in diabetes. *Diabetes Care*, 27 Suppl 1, S79–83.

Morales, E., Valero, M. A., Leon, M., *et al.* (2003). Beneficial effects of weight loss in overweight patients with chronic proteinuric nephropathies. *Am J Kidney Dis*, 41(2), 319–27.

Morigi, M., Buelli, S., Angioletti, S., *et al.* (2005). In response to protein load podocytes reorganize cytoskeleton and modulate endothelin-1 gene: implication for permselective dysfunction of chronic nephropathies. *Am J Pathol*, 166(5), 1309–20.

Morikawa, A., Ishizeki, K., Iwashima, Y., *et al.* (2011). Pioglitazone reduces urinary albumin excretion in renin-angiotensin system inhibitor-treated type 2 diabetic patients with hypertension and microalbuminuria: the APRIME study. *Clin Exp Nephrol*, 15(6), 848–53.

Murakami, R., Murakami, S., Tsushima, R., *et al.* (2008). Glycaemic control and serum intact parathyroid hormone levels in diabetic patients on haemodialysis therapy. *Nephrol Dial Transplant*, 23(1), 315–20.

Nagata, M. and Kriz, W. (1992). Glomerular damage after uninephrectomy in young rats. II. Mechanical stress on podocytes as a pathway to sclerosis. *Kidney Int*, 42(1), 148–60.

Najafian, B., Crosson, J. T., Kim, Y., *et al.* (2006). Glomerulotubular junction abnormalities are associated with proteinuria in type 1 diabetes. *J Am Soc Nephrol*, 17(4 Suppl 2), S53–60.

Najafian, B., Kim, Y., Crosson, J. T., *et al.* (2003). Atubular glomeruli and glomerulotubular junction abnormalities in diabetic nephropathy. *J Am Soc Nephrol*, 14(4), 908–17.

Nakagawa, T. (2007). Uncoupling of the VEGF-endothelial nitric oxide axis in diabetic nephropathy: an explanation for the paradoxical effects of VEGF in renal disease. *Am J Physiol Renal Physiol*, 292(6), F1665–72.

Nakamura, T., Sugaya, T., Kawagoe, Y., *et al.* (2005). Effect of pitavastatin on urinary liver-type fatty acid-binding protein levels in patients with early diabetic nephropathy. *Diabetes Care*, 28(11), 2728–32.

National Institute for Health and Care Excellence (2009). *Type 2 Diabetes: The Management of Type 2 Diabetes*. [Online] <http://www.nice.org.uk/guidance/cg87>

Navaneethan, S. D., Nigwekar, S. U., Sehgal, A. R., *et al.* (2009). Aldosterone antagonists for preventing the progression of chronic kidney disease: a systematic review and meta-analysis. *Clin J Am Soc Nephrol*, 4(3), 542–51.

Navarro, X., Kennedy, W. R., and Sutherland, D. E. (1991). Autonomic neuropathy and survival in diabetes mellitus: effects of pancreas transplantation. *Diabetologia*, 34 Suppl 1, S108–12.

Neal, C. R., Crook, H., Bell, E., *et al.* (2005). Three-dimensional reconstruction of glomeruli by electron microscopy reveals a distinct restrictive urinary subpodocyte space. *J Am Soc Nephrol*, 16(5), 1223–35.

Nelson, R. G., Bennett, P. H., Beck, G. J., *et al.* (1996). Development and progression of renal disease in Pima Indians with non-insulin-dependent diabetes mellitus. Diabetic Renal Disease Study Group. *N Engl J Med*, 335(22), 1636–42.

Nelson, R. G., Knowler, W. C., Pettitt, D. J., *et al.* (1995). Incidence and determinants of elevated urinary albumin excretion in Pima Indians with NIDDM. *Diabetes Care*, 18(2), 182–7.

Nelson, R. G., Meyer, T. W., Myers, B. D., *et al.* (1997). Course of renal disease in Pima Indians with non-insulin-dependent diabetes mellitus. *Kidney Int Suppl*, 63, S45–8.

Nelson, R. G., Pettitt, D. J., Baird, H. R., *et al.* (1993). Pre-diabetic blood pressure predicts urinary albumin excretion after the onset of type 2 (non-insulin-dependent) diabetes mellitus in Pima Indians. *Diabetologia*, 36(10), 998–1001.

Neuhofer, W. and Pittrow, D. (2009). Endothelin receptor selectivity in chronic kidney disease: rationale and review of recent evidence. *Eur J Clin Invest*, 39 Suppl 2, 50–67.

Nguyen, G. and Danser, A. H. (2008). Prorenin and (pro)renin receptor: a review of available data from in vitro studies and experimental models in rodents. *Exp Physiol*, 93(5), 557–63.

Nguyen, G., Delarue, F., Burckle, C., *et al.* (2002). Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest*, 109(11), 1417–27.

Nielsen, F. S., Rossing, P., Gall, M. A., *et al.* (1994). Impact of lisinopril and atenolol on kidney function in hypertensive NIDDM subjects with diabetic nephropathy. *Diabetes*, 43(9), 1108–13.

Nissen, S. E. and Wolski, K. (2007). Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med*, 356(24), 2457–71.

Nissen, S. E. and Wolski, K. (2010). Rosiglitazone revisited: an updated meta-analysis of risk for myocardial infarction and cardiovascular mortality. *Arch Intern Med*, 170(14), 1191–201.

Oberbauer, R., Nenov, V., Weidekamm, C., *et al.* (2001). Reduction in mean glomerular pore size coincides with the development of large shunt pores in patients with diabetic nephropathy. *Exp Nephrol*, 9(1), 49–53.

Ohkubo, Y., Kishikawa, H., Araki, E., *et al.* (1995). Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract*, 28(2), 103–17.

Ohkubo, Y., Kishikawa, H., Araki, E., *et al.* (1995). Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract*, 28(2), 103–17.

Ohtomo, S., Izuhara, Y., Takizawa, S., *et al.* (2007). Thiazolidinediones provide better renoprotection than insulin in an obese, hypertensive type II diabetic rat model. *Kidney Int*, 72(12), 1512–19.

Olsen, S. and Mogensen, C. E. (1996). How often is NIDDM complicated with non-diabetic renal disease? An analysis of renal biopsies and the literature. *Diabetologia*, 39(12), 1638–45.

Onozato, M. L., Tojo, A., Goto, A., *et al.* (2002). Oxidative stress and nitric oxide synthase in rat diabetic nephropathy: effects of ACEI and ARB. *Kidney Int*, 61(1), 186–94.

Opie, L. (1999). *Angiotensin-Converting Enzyme Inhibitors: The Advances Continues* (3rd ed.). Cape Town: University of Cape Town Press.

Orchard, T. J., Chang, Y. F., Ferrell, R. E., *et al.* (2002). Nephropathy in type 1 diabetes: a manifestation of insulin resistance and multiple genetic susceptibilities? Further evidence from the Pittsburgh Epidemiology of Diabetes Complication Study. *Kidney Int*, 62(3), 963–70.

Osterby, R. (1992). Glomerular structural changes in type 1 (insulin-dependent) diabetes mellitus: causes, consequences, and prevention. *Diabetologia*, 35(9), 803–12.

Osterby, R., Asplund, J., Bangstad, H. J., *et al.* (1999). Neovascularization at the vascular pole region in diabetic glomerulopathy. *Nephrol Dial Transplant*, 14(2), 348–52.

Osterby, R. and Nyberg, G. (1987). New vessel formation in the renal corpuscles in advanced diabetic glomerulopathy. *J Diabet Complications*, 1(4), 122–7.

Packham, D. K., Wolfe, R., Reutens, A. T., *et al.* (2012). Sulodexide fails to demonstrate renoprotection in overt type 2 diabetic nephropathy. *J Am Soc Nephrol*, 23(1), 123–30.

Pagtalunan, M. E., Miller, P. L., Jumping-Eagle, S., *et al.* (1997). Podocyte loss and progressive glomerular injury in type II diabetes. *J Clin Invest*, 99(2), 342–8.

Parvanova, A., Remuzzi, G., Benigni, A., *et al.* (2011). *Ameliorating Day- and Night-Time Blood Pressure Control by Combined ECE/NEP Inhibition with Daglutril on top of Losartan*. Poster presented at the Annual Congress of the American Society of Nephrology, Philadelphia, USA.

Parvanova, A. I., Trevisan, R., Iliev, I. P., *et al.* (2006).Insulin resistance and microalbuminuria: a cross-sectional, case-control study of 158 patients with type 2 diabetes and different degrees of urinary albumin excretion. *Diabetes*, 55(5), 1456–62.

Parving, H. H. (1998). Renoprotection in diabetes: genetic and non-genetic risk factors and treatment. *Diabetologia*, 41(7), 745–59.

Parving, H. H. (2000). Blockade of the renin-angiotensin-aldosterone system and renal protection in diabetes mellitus. *J Renin Angiotensin Aldosterone Syst*, 1(1), 30–1.

Parving, H. H., Brenner, B. M., McMurray, J. J., *et al.* (2012). Cardiorenal end points in a trial of aliskiren for type 2 diabetes. *N Engl J Med*, 367(23), 2204–13.

Parving, H. H., Hommel, E., Smidt, U. M. (1988). Protection of kidney function and decrease in albuminuria by captopril in insulin dependent diabetics with nephropathy. *BMJ*, 297(6656), 1086–91.

Parving, H. H. and Hovind, P. (2002). Microalbuminuria in type 1 and type 2 diabetes mellitus: evidence with angiotensin converting enzyme inhibitors and angiotensin II receptor blockers for treating early and preventing clinical nephropathy. *Curr Hypertens Rep*, 4(5), 387–93.

Parving, H. H., Lehnert, H., Brochner-Mortensen, J., *et al.* (2001). The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med*, 345(12), 870–8.

Parving, H. H., Persson, F., Lewis, J. B., *et al.* (2008). Aliskiren combined with losartan in type 2 diabetes and nephropathy. *N Engl J Med*, 358(23), 2433–46.

Passa, P., LeBlanc, H., and Marre, M. (1987). Effects of enalapril in insulin-dependent diabetic subjects with mild to moderate uncomplicated hypertension. *Diabetes Care*, 10(2), 200–4.

Patel, A., Group, A. C., MacMahon, S., *et al.* (2007). Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial. *Lancet*, 370(9590), 829–40.

Patel, A., MacMahon, S., Chalmers, J., *et al.* (2008). Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med*, 358(24), 2560–72.

Paueksakon, P., Revelo, M. P., Ma, L. J., *et al.* (2002). Microangiopathic injury and augmented PAI-1 in human diabetic nephropathy. *Kidney Int*, 61(6), 2142–8.

Pedersen, M. M., Schmitz, A., Pedersen, E. B., *et al.* (1988). Acute and long-term renal effects of angiotensin converting enzyme inhibition in normotensive, normoalbuminuric insulin-dependent diabetic patients. *Diabet Med*, 5(6), 562–9.

Pedrini, M. T., Levey, A. S., Lau, J., *et al.* (1996). The effect of dietary protein restriction on the progression of diabetic and nondiabetic renal diseases: a meta-analysis. *Ann Intern Med*, 124(7), 627–32.

Pelle, G., Shweke, N., Duong Van Huyen, J. P., *et al.* (2011). Systemic and kidney toxicity of intraocular administration of vascular endothelial growth factor inhibitors. *Am J Kidney Dis*, 57(5), 756–9.

Pergola, P. E., Krauth, M., Huff, J. W., *et al.* (2011). Effect of bardoxolone methyl on kidney function in patients with T2D and Stage 3b-4 CKD. *Am J Nephrol*, 33(5), 469–76.

Perico, N., Amuchastegui, S. C., Colosio, V., *et al.* (1994). Evidence that an angiotensin-converting enzyme inhibitor has a different effect on glomerular injury according to the different phase of the disease at which the treatment is started. *J Am Soc Nephrol*, 5(4), 1139–46.

Perkins, B. A., Ficociello, L. H., Ostrander, B. E., *et al.* (2007). Microalbuminuria and the risk for early progressive renal function decline in type 1 diabetes. *J Am Soc Nephrol*, 18(4), 1353–61.

Perkins, B. A. (2012). Intensive therapy and GFR in type 1 diabetes. *N Engl J Med*, 366(9), 856–8.

Persson, F., Rossing, P., Hovind, P., *et al.* (2008). Endothelial dysfunction and inflammation predict development of diabetic nephropathy in the Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria (IRMA 2) study. *Scand J Clin Lab Invest*, 68(8), 731–8.

Persson, F., Rossing, P., Hovind, P., *et al.* (2006). Irbesartan treatment reduces biomarkers of inflammatory activity in patients with type 2 diabetes and microalbuminuria: an IRMA 2 substudy. *Diabetes*, 55(12), 3550–5.

Persson, F., Rossing, P., Reinhard, H., *et al.* (2009). Renal effects of aliskiren compared with and in combination with irbesartan in patients with type 2 diabetes, hypertension, and albuminuria. *Diabetes Care*, 32(10), 1873–9.

Pfeffer, M. A., Burdmann, E. A., Chen, C. Y., *et al.* A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med*, 361(21), 2019–32.

Piecha, G., Koleganova, N., Gross, M. L., *et al.* (2008). Regression of glomerulosclerosis in subtotally nephrectomized rats: effects of monotherapy with losartan, spironolactone, and their combination. *Am J Physiol Renal Physiol*, 295(1), F137–44.

Pohl, M. A., Blumenthal, S., Cordonnier, D. J., *et al.* (2005). Independent and additive impact of blood pressure control and angiotensin II receptor blockade on renal outcomes in the irbesartan diabetic nephropathy trial: clinical implications and limitations. *J Am Soc Nephrol*, 16(10), 3027–37.

Pop-Busui, R., Roberts, L., Pennathur, S., *et al.* (2010). The management of diabetic neuropathy in CKD. *Am J Kidney Dis*, 55(2), 365–85.

Poulsen, P. L., Hansen, K. W., and Mogensen, C. E. (1994). Ambulatory blood pressure in the transition from normo- to microalbuminuria. A longitudinal study in IDDM patients. *Diabetes*, 43(10), 1248–53.

Poulter, N. R. (2009). Blood pressure and glucose control in subjects with diabetes: new analyses from ADVANCE. *J Hyperten*, 27 Suppl 1, S3–8.

Powers, D. R. and Wallin, J. D. (1998). End-stage renal disease in specific ethnic and racial groups: risk factors and benefits of antihypertensive therapy. *Arch Intern Med*, 158(7), 793–800.

Premaratne, E., MacIsaac, R. J., Finch, S., *et al.* (2008). Serial measurements of cystatin C are more accurate than creatinine-based methods in detecting declining renal function in type 1 diabetes. *Diabetes Care*, 31(5), 971–3.

Prisant, L. M., Weir, M. R., Papademetriou, V., *et al.* (1995). Low-dose drug combination therapy: an alternative first-line approach to hypertension treatment. *Am Heart J*, 130(2), 359–66.

Quinn, M., Angelico, M. C., Warram, J. H., *et al.* (1996). Familial factors determine the development of diabetic nephropathy in patients with IDDM. *Diabetologia*, 39(8), 940–5.

Rahmoune, H., Thompson, P. W., Ward, J. M., *et al.* (2005). Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. *Diabetes*, 54(12), 3427–34.

Raij, L. (2005). The pathophysiologic basis for blocking the renin-angiotensin system in hypertensive patients with renal disease. *Am J Hypertens*, 18(4 Pt 2), 95S–9S.

Ranpuria, R., Hall, M., Chan, C. T., *et al.* (2008). Heart rate variability (HRV) in kidney failure: measurement and consequences of reduced HRV. *Nephrol Dial Transplant*, 23(2), 444–9.

Rathmann, W., Ziegler, D., Jahnke, M., *et al.* (1993). Mortality in diabetic patients with cardiovascular autonomic neuropathy. *Diabet Med*, 10(9), 820–4.

Ravid, M., Brosh, D., Ravid-Safran, D., *et al.* (1998). Main risk factors for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. *Arch Intern Med*, 158(9), 998–1004.

Ravid, M., Lang, R., Rachmani, R., *et al.* (1996). Long-term renoprotective effect of angiotensin-converting enzyme inhibition in non-insulin-dependent diabetes mellitus. A 7-year follow-up study. *Arch Intern Med*, 156(3), 286–9.

Reboldi, G., Gentile, G., Angeli, F., *et al.* (2010). Blood pressure lowering in the oldest old. *J Hyperten*, 28(7), 1373–6.

Reboldi, G., Gentile, G., Angeli, F., *et al.* (2009). Choice of ACE inhibitor combinations in hypertensive patients with type 2 diabetes: update after recent clinical trials. *Vasc Health Risk Manag*, 5(1), 411–27.

Reboldi, G., Gentile, G., Angeli, F., *et al.* (2011). Optimal therapy in hypertensive subjects with diabetes mellitus. *Curr Atheroscler Rep*, 13(2), 176–85.

Redon, J., Mancia, G., Sleight, P., *et al.* (2012). Safety and efficacy of low blood pressures among patients with diabetes: subgroup analyses from the ONTARGET (ONgoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial). *J Am Coll Cardiol*, 59(1), 74–83.

Reitsma, S., Slaaf, D. W., Vink, H., *et al.* (2007). The endothelial glycocalyx: composition, functions, and visualization. *Pflugers Arch*, 454(3), 345–59.

Remuzzi, A., Viberti, G., Ruggenenti, P., *et al.* (1990). Glomerular response to hyperglycemia in human diabetic nephropathy. *Am J Physiol*, 259(4 Pt 2), F545–52.

Remuzzi, A. and Remuzzi, G. (1990). Assessment of glomerular size-selective function with fractional clearance of neutral dextran. *J Lab Clin Med*, 132(5), 360–2.

Remuzzi, A. and Remuzzi, G. (1994). Glomerular perm-selective function. *Kidney Int*, 45(2), 398–402.

Remuzzi, G., Benigni, A., and Remuzzi, A. (2006). Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J Clin Invest*, 116(2), 288–96.

Remuzzi, G., Perico, N., and Benigni, A. (2002a). New therapeutics that antagonize endothelin: promises and frustrations. *Nat Rev Drug Discov*, 1(12), 986–1001.

Remuzzi, G., Schieppati, A., and Ruggenenti, P. (2002b). Clinical practice. Nephropathy in patients with type 2 diabetes. *N Engl J Med*, 346(15), 1145–51.

Remuzzi, G. and Bertani, T. (1990). Is glomerulosclerosis a consequence of altered glomerular permeability to macromolecules? *Kidney Int*, 38(3), 384–94.

Remuzzi, G. and Bertani, T. (1998). Pathophysiology of progressive nephropathies. *N Engl J Med*, 339(20), 1448–56.

Remuzzi, G. and Ruggenenti, P. (2006). Overview of randomised trials of ACE inhibitors. *Lancet*, 368(9535), 555–6.

Riddle, M. C. (2010). Counterpoint: Intensive glucose control and mortality in ACCORD--still looking for clues. *Diabetes Care*, 33(12), 2722–4.

Riser, B. L. and Cortes, P. (2001). Connective tissue growth factor and its regulation: a new element in diabetic glomerulosclerosis. *Ren Fail*, 23(3–4), 459–70.

Ritz, E., Laville, M., Bilous, R. W., *et al.* (2007). Target level for hemoglobin correction in patients with diabetes and CKD: primary results of the Anemia Correction in Diabetes (ACORD) Study. *Am J Kidney Dis*, 49(2), 194–207.

Rizkalla, B., Forbes, J. M., Cao, Z., *et al.* (2005). Temporal renal expression of angiogenic growth factors and their receptors in experimental diabetes: role of the renin-angiotensin system. *J Hyperten*, 23(1), 153–64.

Robertson, L., Waugh, N., and Robertson, A. (2007). Protein restriction for diabetic renal disease. *Cochrane Database Syst Rev*, 4, CD002181.

Rognant, N., Lemoine, S., Laville, M., *et al.* (2011). Performance of the chronic kidney disease epidemiology collaboration equation to estimate glomerular filtration rate in diabetic patients. *Diabetes Care*, 34(6), 1320–2.

Roscioni, S. S., de Zeeuw, D., Hellemons, M. E., *et al.* (2013). A urinary peptide biomarker set predicts worsening of albuminuria in type 2 diabetes mellitus. *Diabetologia*, 56(2), 259–67.

Rosolowsky, E. T., Skupien, J., Smiles, A. M., *et al.* (2011). Risk for ESRD in type 1 diabetes remains high despite renoprotection. *J Am Soc Nephrol*, 22(3), 545–53.

Rossi, G. P. (2006). Aldosterone breakthrough during RAS blockade: a role for endothelins and their antagonists? *Curr Hypertens Rep*, 8(3), 262–8.

Rossing, K., Christensen, P. K., Hovind, P., *et al.* (2004). Progression of nephropathy in type 2 diabetic patients. *Kidney Int*, 66(4), 1596–605.

Rossing, P., Hansen, B. V., Nielsen, F. S., *et al.* (1996a). Fish oil in diabetic nephropathy. *Diabetes Care*, 19(11), 1214–19.

Rossing, P., Hougaard, P., Borch-Johnsen, K., *et al.* (1996b). Predictors of mortality in insulin dependent diabetes: 10 year observational follow up study. *BMJ*, 313(7060), 779–84.

Rossing, P., Hougaard, P., and Parving, H. H. (2002). Risk factors for development of incipient and overt diabetic nephropathy in type 1 diabetic patients: a 10-year prospective observational study. *Diabetes Care*, 25(5), 859–64.

Roszer, T. and Ricote, M. (2010). PPARs in the renal regulation of systemic blood pressure. *PPAR Res*, 2010, 698730.

Rubio-Guerra, A. F., Arceo-Navarro, A., Vargas-Ayala, G., *et al.* (2004). The effect of trandolapril and its fixed-dose combination with verapamil on proteinuria in normotensive adults with type 2 diabetes. *Diabetes Care*, 27(7), 1688–91.

Ruggenenti, P., Bettinaglio, P., Pinares, F., *et al.* (2008a). Angiotensin converting enzyme insertion/deletion polymorphism and renoprotection in diabetic and nondiabetic nephropathies. *Clin J Am Soc Nephrol*, 3(5), 1511–25.

Ruggenenti, P., Cravedi, P., Chianca, A., *et al.* (2012a). Rituximab in idiopathic membranous nephropathy. *J Am Soc Nephrol*, 23(8), 1416–25.

Ruggenenti, P., Cravedi, P., and Remuzzi, G. (2009). Proteinuria: increased angiotensin-receptor blocking is not the first option. *Nat Rev Nephrol*, 5(7), 367–8.

Ruggenenti, P., Cravedi, P., and Remuzzi, G. (2010c). The RAAS in the pathogenesis and treatment of diabetic nephropathy. *Nat Rev Nephrol*, 6(6), 319–30.

Ruggenenti, P., Cravedi, P., and Remuzzi, G. (2012f). Mechanisms and treatment of CKD. *J Am Soc Nephrol*, 23(12), 1917–28.

Ruggenenti, P., Fassi, A., Ilieva, A. P., *et al.* (2011a). Effects of verapamil added-on trandolapril therapy in hypertensive type 2 diabetes

patients with microalbuminuria: the BENEDICT-B randomized trial. *J Hypertens*, 29(2), 207–16.
Ruggenenti, P., Fassi, A., Ilieva, A. P., *et al.* (2004). Preventing microalbuminuria in type 2 diabetes. *N Engl J Med*, 351(19), 1941–51.
Ruggenenti, P., Gambara, V., Perna, A., *et al.* (1998). The nephropathy of non-insulin-dependent diabetes: predictors of outcome relative to diverse patterns of renal injury. *J Am Soc Nephrol*, 9(12), 2336–43.
Ruggenenti, P., Gaspari, F., Cannata, A., *et al.* (2012e). Measuring and estimating GFR and treatment effect in ADPKD patients: results and implications of a longitudinal cohort study. *PloS One*, 7(2), e32533.
Ruggenenti, P., Iliev, I., Filipponi, M., *et al.* (2010a). Effect of trandolapril on regression of retinopathy in hypertensive patients with type 2 diabetes: a prespecified analysis of the benedict trial. *J Ophthalmol*, 106384.
Ruggenenti, P., Lauria, G., Iliev, I. P., *et al.* (2011b). Effects of manidipine and delapril in hypertensive patients with type 2 diabetes mellitus: The Delapril and Manidipine for Nephroprotection in Diabetes (DEMAND) Randomized Clinical Trial. *Hypertension*, 58(5), 776–83.
Ruggenenti, P., Perna, A., Ganeva, M., *et al.* (2006). Impact of blood pressure control and angiotensin-converting enzyme inhibitor therapy on new-onset microalbuminuria in type 2 diabetes: a post hoc analysis of the BENEDICT trial. *J Am Soc Nephrol*, 17(12), 3472–81.
Ruggenenti, P., Perna, A., Loriga, G., *et al.* (2005). Blood-pressure control for renoprotection in patients with non-diabetic chronic renal disease (REIN-2), multicentre, randomised controlled trial. *Lancet*, 365(9463), 939–46.
Ruggenenti, P., Perna, A., Tonelli, M., *et al.* (2010b). Effects of add-on fluvastatin therapy in patients with chronic proteinuric nephropathy on dual renin-angiotensin system blockade: the ESPLANADE trial. *Clin J Am Soc Nephrol*, 5(11), 1928–38.
Ruggenenti, P., Perticucci, E., Cravedi, P., *et al.* (2008b). Role of remission clinics in the longitudinal treatment of CKD. *J Am Soc Nephrol*, 19(6), 1213–24.
Ruggenenti, P., Porrini, E., Motterlini, N., *et al.* (2012d). Measurable urinary albumin predicts cardiovascular risk among normoalbuminuric patients with type 2 diabetes. *J Am Soc Nephrol*, 23(10), 1717–24.
Ruggenenti, P., Porrini, E. L., Gaspari, F., *et al.* (2012c). Glomerular hyperfiltration and renal disease progression in type 2 diabetes. *Diabetes Care*, 35(10), 2061–8.
Ruggenenti, P., Ruggiero, B., Cravedi, P., *et al.* (2012b). Rituximab in steroid-dependent or multirelapsing nephrotic syndrome of adults and children: results from the NEMO Trial. *J Am Soc Nephrol*, 23, 723A.
Ruggenenti, P. and Remuzzi, G. (2009). Proteinuria: is the ONTARGET renal substudy actually off target? *Nat Rev Nephrol*, 5(8), 436–7.
Ruggenenti, P. and Remuzzi, G. (2006). Time to abandon microalbuminuria? *Kidney Int*, 70(7), 1214–22.
Russo, L. M., Sandoval, R. M., Campos, S. B., *et al.* (2009). Impaired tubular uptake explains albuminuria in early diabetic nephropathy. *J Am Soc Nephrol*, 20(3), 489–94.
Rutledge, J. C., Ng, K. F., Aung, H. H., *et al.* (2010). Role of triglyceride-rich lipoproteins in diabetic nephropathy. *Nat Rev Nephrol*, 6(6), 361–70.
Rutter, M. K., Prais, H. R., Charlton-Menys, V., *et al.* (2011). Protection Against Nephropathy in Diabetes with Atorvastatin (PANDA): a randomized double-blind placebo-controlled trial of high- vs. low-dose atorvastatin(1). *Diabet Med*, 28(1), 100–8.
Ryan, K. K., Li, B., Grayson, B. E., *et al.* (2011). A role for central nervous system PPAR-gamma in the regulation of energy balance. *Nat Med*, 17(5), 623–6.
Salmon, A. H., Neal, C. R., and Harper, S. J. (2009a). New aspects of glomerular filtration barrier structure and function: five layers (at least) not three. *Curr Opin Nephrol Hypertens*, 18(3), 197–205.
Salmon, A. H., Neal, C. R., Sage, L. M., *et al.* (2009b). Angiopoietin-1 alters microvascular permeability coefficients in vivo via modification of endothelial glycocalyx. *Cardiovasc Res* 83(1), 24–33.
Salmon, A. H., Toma, I., Sipos, A., *et al.* (2007). Evidence for restriction of fluid and solute movement across the glomerular capillary wall by the subpodocyte space. *Am J Physiol Renal Physiol*, 293(6), F1777–86.
Santilli, F., Cipollone, F., Mezzetti, A., *et al.* (2004). The role of nitric oxide in the development of diabetic angiopathy. *HormMetab Res*, 36(5), 319–35.
Satchell, S. C., Anderson, K. L., and Mathieson, P. W. (2004). Angiopoietin 1 and vascular endothelial growth factor modulate human glomerular endothelial cell barrier properties. *J Am Soc Nephrol*, 15(3), 566–74.
Satchell, S. C., Harper, S. J., Tooke, J. E., *et al.* (2002). Human podocytes express angiopoietin 1, a potential regulator of glomerular vascular endothelial growth factor. *J Am Soc Nephrol*, 13(2), 544–50.
Satchell, S. C. and Tooke, J. E. (2008). What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium? *Diabetologia*, 51(5), 714–25.
Sawicki, P. T., Didjurgeit, U., Muhlhauser, I., *et al.* (1994). Smoking is associated with progression of diabetic nephropathy. *Diabetes Care*, 17(2), 126–31.
Schena, F. P. (1994). Domenico Cotugno and his interest in proteinuria. *Am J Nephrol*,14(4–6), 325–9.
Schmieder, R. E., Mann, J. F., Schumacher, H., *et al.* (2011). Changes in albuminuria predict mortality and morbidity in patients with vascular disease. *J Am Soc Nephrol*, 22(7), 1353–64.
Schrier, R. W., Estacio, R. O., Esler, A., *et al.* (2002). Effects of aggressive blood pressure control in normotensive type 2 diabetic patients on albuminuria, retinopathy and strokes. *Kidney Int*, 61(3), 1086–97.
Schultz, C. J., Konopelska-Bahu, T., Dalton, R. N., *et al.* (1999). Microalbuminuria prevalence varies with age, sex, and puberty in children with type 1 diabetes followed from diagnosis in a longitudinal study. Oxford Regional Prospective Study Group. *Diabetes Care*, 22(3), 495–502.
Schulz, E., Jansen, T., Wenzel, P., *et al.* (2008). Nitric oxide, tetrahydrobiopterin, oxidative stress, and endothelial dysfunction in hypertension. *Antioxid Redox Signal*, 10(6), 1115–26.
Seaquist, E. R., Goetz, F. C., Rich, S., *et al.* (1989). Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. *N Engl J Med*, 320(18), 1161–5.
Sharma, K., Cook, A., Smith, M., *et al.* (2005). TGF-beta impairs renal autoregulation via generation of ROS. *Am J Physiol Renal Physiol*, 288(5), F1069–77.
Sharma, K., Ix, J. H., Mathew, A. V., *et al.* (2011). Pirfenidone for diabetic nephropathy. *J Am Soc Nephrol*, 22(6), 1144–51.
Shaw, J. E., Sicree, R. A., and Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract*, 87(1), 4–14.
Shichiri, M., Kishikawa, H., Ohkubo, Y., *et al.* (2000). Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. *Diabetes Care*, 23 Suppl 2, B21–9.
Silveiro, S. P., Araujo, G. N., Ferreira, M. N., *et al.* (2011). Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation pronouncedly underestimates glomerular filtration rate in type 2 diabetes. *Diabetes Care*, 34(11), 2353–5.
Sison, K., Eremina, V., Baelde, H., *et al.* (2010). Glomerular structure and function require paracrine, not autocrine, VEGF-VEGFR-2 signaling. *J Am Soc Nephrol*, 21(10), 1691–701.
Sjolie, A. K., Klein, R., Porta, M., *et al.* (2008). Effect of candesartan on progression and regression of retinopathy in type 2 diabetes (DIRECT-Protect 2), a randomised placebo-controlled trial. *Lancet*, 372(9647), 1385–93.
Smith, A. C., Toto, R., and Bakris, G. L. (1998). Differential effects of calcium channel blockers on size selectivity of proteinuria in diabetic glomerulopathy. *Kidney Int*, 54(3), 889–96.
Soni, S. S., Gowrishankar, S., Kishan, A. G., *et al.* (2006). Non diabetic renal disease in type 2 diabetes mellitus. *Nephrology*, 11(6), 533–7.
Steffes, M. W., Osterby, R., Chavers, B., *et al.* (1989). Mesangial expansion as a central mechanism for loss of kidney function in diabetic patients. *Diabetes*, 38(9), 1077–81.
Steffes, M. W., Schmidt, D., McCrery, R., *et al.* (2001). Glomerular cell number in normal subjects and in type 1 diabetic patients. *Kidney Int*, 59(6), 2104–13.

Stehouwer, C. D. and Smulders, Y. M. (2006). Microalbuminuria and risk for cardiovascular disease: Analysis of potential mechanisms. *J Am Soc Nephrol*, 17(8), 2106–11.

Stehouwer, C. D. (2004). Endothelial dysfunction in diabetic nephropathy: state of the art and potential significance for non-diabetic renal disease. *Nephrol Dial Transplant*, 19(4), 778–81.

Stella, P., Ellis, D., Maser, R. E., *et al.* (2000). Cardiovascular autonomic neuropathy (expiration and inspiration ratio) in type 1 diabetes. Incidence and predictors. *J Diabetes Complications*, 14(1), 1–6.

Stenvinkel, P., Heimburger, O., and Jogestrand, T. (2002). Elevated interleukin-6 predicts progressive carotid artery atherosclerosis in dialysis patients: association with Chlamydia pneumoniae seropositivity. *Am J Kidney Dis*, 39(2), 274–82.

Stephenson, J. M. and Fuller, J. H. (1994). Microalbuminuria is not rare before 5 years of IDDM. EURODIAB IDDM Complications Study Group and the WHO Multinational Study of Vascular Disease in Diabetes Study Group. *J Diabetes Complications*, 8(3), 166–73.

Stevens, L. A., Djurdjev, O., Cardew, S., *et al.* (2004). Calcium, phosphate, and parathyroid hormone levels in combination and as a function of dialysis duration predict mortality: evidence for the complexity of the association between mineral metabolism and outcomes. *J Am Soc Nephrol*, 15(3), 770–9.

Stout, L. C., Kumar, S., and Whorton, E. B. (1994). Insudative lesions--their pathogenesis and association with glomerular obsolescence in diabetes: a dynamic hypothesis based on single views of advancing human diabetic nephropathy. *Hum Pathol*, 25(11), 1213–27.

Struthers, A., Krum, H., and Williams, G. H. (2008). A comparison of the aldosterone-blocking agents eplerenone and spironolactone. *Clin Cardiol*, 31(4), 153–8.

Sung, S. H., Ziyadeh, F. N., Wang, A., *et al.* (2006). Blockade of vascular endothelial growth factor signaling ameliorates diabetic albuminuria in mice. *J Am Soc Nephrol*, 17(11), 3093–104.

Suzuki, K., Babazono, T., Murata, H., *et al.* (2005). Clinical significance of urinary liver-type fatty acid-binding protein in patients with diabetic nephropathy. *Diabetes Care*, 28(8), 2038–9.

Tabatabai, N. M., Sharma, M., Blumenthal, S. S., *et al.* (2009). Enhanced expressions of sodium-glucose cotransporters in the kidneys of diabetic Zucker rats. *Diabetes Res Clin Pract*, 83(1), e27–30.

Taguma, Y., Kitamoto, Y., Futaki, G., *et al.* (1985). Effect of captopril on heavy proteinuria in azotemic diabetics. *N Engl J Med*, 313(26), 1617–20.

Takaichi, K., Takemoto, F., Ubara, Y., *et al.* (2007). Analysis of factors causing hyperkalemia. *Intern Med*, 46(12), 823–9.

Tang, S. C., Leung, J. C., Chan, L. Y., *et al.* (2006). Activation of tubular epithelial cells in diabetic nephropathy and the role of the peroxisome proliferator-activated receptor-gamma agonist. *J Am Soc Nephrol*, 17(6), 1633–43.

Tanner, G. A. (2009). Glomerular sieving coefficient of serum albumin in the rat: a two-photon microscopy study. *AmJ Physiol Renal Physiol*, 296(6), F1258–65.

Taylor, A. A. (2004). Combination drug treatment of hypertension: have we come full circle? *Curr Cardiol Rep*, 6(6), 421–6.

Tervaert, T. W., Mooyaart, A. L., Amann, K., *et al.* (2010). Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol*, 21(4), 556–63.

Tesfaye, S., Boulton, A. J., Dyck, P. J., *et al.* (2010). Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*, 33(10), 2285–93.

Tesfaye, S., Stevens, L. K., Stephenson, J. M., *et al.* (1996). Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: the EURODIAB IDDM Complications Study. *Diabetologia*, 39(11), 1377–84.

The ACE Inhibitors in Diabetic Nephropathy Trialist Group (2001). Should all patients with type 1 diabetes mellitus and microalbuminuria receive angiotensin-converting enzyme inhibitors? A meta-analysis of individual patient data. *Ann Intern Med*, 134, 370 –9.

The Consensus Committee of the American Autonomic Society and the American Academy of Neurology (1996). Consensus statement on the definition of orthostatic hypotension, pure autonomic failure, and multiple system atrophy. *Neurology*, 46(5), 1470.

The Diabetes Control and Complications Trial Research Group (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*, 329(14), 977–86.

The Diabetes Control and Complications Trial Research Group (1995). Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial. *Kidney Int*, 47(6), 1703–20.

The Diabetes Control and Complications Trial Research Group (1998). The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). *Diabetologia*, 41(4), 416–23.

The Microalbuminuria Collaborative Study Group (1999). Predictors of the development of microalbuminuria in patients with type 1 diabetes mellitus: a seven-year prospective study. *DiabetMed*, 16(11), 918–25.

Thomas, M. (2012). A preliminary evaluation of bardoxolone methyl for the treatment of diabetic nephropathy. *Expert Opin Drug Metab Toxicol*, 8(8), 1015–22.

Thomas, M. C., MacIsaac, R. J., Tsalamandris, C., *et al.* (2003). Unrecognized anemia in patients with diabetes: a cross-sectional survey. *Diabetes Care*, 26(4), 1164–9.

Thomas, M. C., MacIsaac, R. J., Tsalamandris, C., *et al.* (2004a). Anemia in patients with type 1 diabetes. *J Clin Endocrinol Metab*, 89(9), 4359–63.

Thomas, M. C., MacIsaac, R. J., Tsalamandris, C., *et al.* (2004b). The burden of anaemia in type 2 diabetes and the role of nephropathy: a cross-sectional audit. *Nephrol Dial Transplant*, 19(7), 1792–7.

Thomas, M., Tsalamandris, C., MacIsaac, R., *et al.* (2005). Anaemia in diabetes: an emerging complication of microvascular disease. *Curr Diabetes Rev*, 1(1), 107–26.

Thomas, S., Vanuystel, J., Gruden, G., *et al.* (2000). Vascular endothelial growth factor receptors in human mesangium in vitro and in glomerular disease. *J Am Soc Nephrol*, 11(7), 1236–43.

Thorens, B. and Mueckler, M. (2010). Glucose transporters in the 21st Century. *Am J Physiol Endocrinol Metab*, 298(2), E141–5.

Titan, S. M., Vieira, J. J., Dominguez, W. V., *et al.* (2011). ACEI and ARB combination therapy in patients with macroalbuminuric diabetic nephropathy and low socioeconomic level: a double-blind randomized clinical trial. *Clin Nephrol*, 76(4), 273–83.

Tiwari, S., Halagappa, V. K., Riazi, S., *et al.* (2007). Reduced expression of insulin receptors in the kidneys of insulin-resistant rats. *J Am Soc Nephrol*, 18(10), 2661–71.

Tonolo, G., Velussi, M., Brocco, E., *et al.* (2006). Simvastatin maintains steady patterns of GFR and improves AER and expression of slit diaphragm proteins in type II diabetes. *Kidney Int*, 70(1), 177–86.

Torffvit, O. and Rippe, B. (1999). Size and charge selectivity of the glomerular filter in patients with insulin-dependent diabetes mellitus: urinary immunoglobulins and glycosaminoglycans. *Nephron* 83(4), 301–7.

Toyoda, M., Najafian, B., Kim, Y., *et al.* (2007). Podocyte detachment and reduced glomerular capillary endothelial fenestration in human type 1 diabetic nephropathy. *Diabetes*, 56(8), 2155–60.

Trevisan, R., Bruttomesso, D., Vedovato, M., *et al.* (1998). Enhanced responsiveness of blood pressure to sodium intake and to angiotensin II is associated with insulin resistance in IDDM patients with microalbuminuria. *Diabetes* 47(8), 1347–53.

Tsao, T., Kornblau, S., Safe, S., *et al.* (2010). Role of peroxisome proliferator-activated receptor-gamma and its coactivator DRIP205 in cellular responses to CDDO (RTA-401) in acute myelogenous leukemia. *Cancer Res*, 70(12), 4949–60.

Tuomilehto, J., Borch-Johnsen, K., Molarius, A., *et al.* (1998). Incidence of cardiovascular disease in Type 1 (insulin-dependent) diabetic subjects with and without diabetic nephropathy in Finland. *Diabetologia*, 41(7), 784–90.

Tuttle, K. R., Bakris, G. L., Toto, R. D., *et al.* (2005). The effect of ruboxistaurin on nephropathy in type 2 diabetes. *Diabetes Care*, 28(11), 2686–90.

UK Prospective Diabetes Study Group (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*, 352(9131), 837–53.

UKPDS Study Group (1998). Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. *BMJ*, 317(7160), 703–13.

United States Renal Data System (2010). *USRDS 2010 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States*. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. <http://www.usrds.org/>

Vallon, V. (2011). The proximal tubule in the pathophysiology of the diabetic kidney. *Am J Physiol Regul Integr Comp Physiol*, 300(5), R1009–22.

Van den Meiracker, A. H., Baggen, R. G., Pauli, S., *et al.* (2006). Spironolactone in type 2 diabetic nephropathy: Effects on proteinuria, blood pressure and renal function. *J Hyperten*, 24(11), 2285–92.

Van Dieren, S., Beulens, J. W., van der Schouw, Y. T., *et al.* (2010). The global burden of diabetes and its complications: an emerging pandemic. *Eur J Cardiovasc Prev Rehabil*, 17(1 suppl), S3–8.

Vanholder, R., De Smet, R., Hsu, C., *et al.* (1994). Uremic toxicity: the middle molecule hypothesis revisited. *Semin Nephrol*, 14(3), 205–18.

Vedovato, M., Lepore, G., Coracina, A., *et al.* (2004). Effect of sodium intake on blood pressure and albuminuria in type 2 diabetic patients: the role of insulin resistance. *Diabetologia*, 47(2), 300–3.

Vegter, S., Perna, A., Postma, M. J., *et al.* (2012). Sodium intake, ACE inhibition, and progression to ESRD. *J Am Soc Nephrol*, 23(1), 165–73.

Velussi, M., Brocco, E., Frigato, F., *et al.* (1996). Effects of cilazapril and amlodipine on kidney function in hypertensive NIDDM patients. *Diabetes*, 45(2), 216–22.

Verdecchia, P., Gentile, G., Angeli, F., *et al.* (2010). Influence of blood pressure reduction on composite cardiovascular endpoints in clinical trials. *J Hyperten*, 28(7), 1356–65.

Veron, D., Reidy, K. J., Bertuccio, C., *et al.* (2010). Overexpression of VEGF-A in podocytes of adult mice causes glomerular disease. *Kidney Int*, 77(11), 989–99.

Vervoort, G., Veldman, B., Berden, J. H., *et al.* (2005). Glomerular hyperfiltration in type 1 diabetes mellitus results from primary changes in proximal tubular sodium handling without changes in volume expansion. *Eur J Clin Invest*, 35(5), 330–6.

Vestbo, E., Damsgaard, E. M., Froland, A., *et al.* (1995). Urinary albumin excretion in a population based cohort. *Diabet Med*, 12(6), 488–93.

Viberti, G., Mogensen, C. E., Groop, L. C., *et al.* (1994). Effect of captopril on progression to clinical proteinuria in patients with insulin-dependent diabetes mellitus and microalbuminuria. European Microalbuminuria Captopril Study Group. *JAMA*, 271(4), 275–9.

Viberti, G., Wheeldon, N. M., and MicroAlbuminuria Reduction With VALsartan (MARVAL) Study Investigators (2002). Microalbuminuria reduction with valsartan in patients with type 2 diabetes mellitus: a blood pressure-independent effect. *Circulation*, 106(6), 672–8.

Viberti, G. C., Hill, R. D., Jarrett, R. J., *et al.* (1982). Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet*, 1(8287), 1430–2.

Vinik, A. I. and Ziegler, D. (2007). Diabetic cardiovascular autonomic neuropathy. *Circulation* 115(3), 387–97.

Vogt, L., Chiurchiu, C., Chadha-Boreham, H., *et al.* (2010). Effect of the urotensin receptor antagonist palosuran in hypertensive patients with type 2 diabetic nephropathy. *Hypertension*, 55(5), 1206–9.

Voulgari, C., Katsilambros, N., and Tentolouris, N. (2011). Smoking cessation predicts amelioration of microalbuminuria in newly diagnosed type 2 diabetes mellitus: a 1-year prospective study. *Metabolism*, 60(10), 1456–64.

Wachtell, K., Ibsen, H., Olsen, M. H., *et al.* (2003). Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study. *Ann Intern Med*, 139(11), 901–6.

Waldherr, R., Ilkenhans, C., and Ritz, E. (1992). How frequent is glomerulonephritis in diabetes mellitus type II? *Clin Nephrol*, 37(6), 271–3.

Wang, C. H., Li, F., Hiller, S., *et al.* (2011). A modest decrease in endothelial NOS in mice comparable to that associated with human NOS3 variants exacerbates diabetic nephropathy. *Proc Natl Acad Sci U S A*, 108(5), 2070–5.

Wang, F., Fang, Q., Yu, N., *et al.* (2012). Association between genetic polymorphism of the angiotensin-converting enzyme and diabetic nephropathy: a meta-analysis comprising 26,580 subjects. *J Renin Angiotensin Aldosterone Syst*, 13(1), 161–74.

Weber, M. A., Bakris, G. L., Jamerson, K., *et al.* (2010). Cardiovascular events during differing hypertension therapies in patients with diabetes. *J Am Coll Cardiol*, 56(1), 77–85.

Weening, J. J. (2004). Advancing nephrology around the globe: an invitation to contribute. *J Am Soc Nephrol*, 15(10), 2761–2.

Weigert, C., Brodbeck, K., Klopfer, K., *et al.* (2002). Angiotensin II induces human TGF-beta 1 promoter activation: similarity to hyperglycaemia. *Diabetologia*, 45(6), 890–8.

Weil, E. J., Lemley, K. V., Mason, C. C., *et al.* (2012). Podocyte detachment and reduced glomerular capillary endothelial fenestration promote kidney disease in type 2 diabetic nephropathy. *Kidney Int*, 82(9), 1010–17.

Weinbaum, S., Tarbell, J. M., and Damiano, E. R. (2007). The structure and function of the endothelial glycocalyx layer. *Annu Rev Biomed Eng*, 9, 121–67.

Weinberger, M. H. (1985). Blood pressure and metabolic responses to hydrochlorothiazide, captopril, and the combination in black and white mild-to-moderate hypertensive patients. *J Cardiovasc Pharmacol*, 7 Suppl 1, S52–5.

Weir, M. R. (1998). Impact of salt intake on blood pressure and proteinuria in diabetes: importance of the renin-angiotensin system. *MinerElectrolyte Metab* 24(6), 438–45.

Weir, M. R. (2007). Targeting mechanisms of hypertensive vascular disease with dual calcium channel and renin-angiotensin system blockade. *J Hum Hypertens*, 21(10), 770–9.

Wenzel, R. R., Littke, T., Kuranoff, S., *et al.* (2009). Avosentan reduces albumin excretion in diabetics with macroalbuminuria. *J Am Soc Nephrol*, 20(3), 655–64.

White, K. E. and Bilous, R. W. (2004). Structural alterations to the podocyte are related to proteinuria in type 2 diabetic patients. *Nephrol Dial Transplant*, 19(6), 1437–40.

White, K. E. and Bilous, R. W. (2000). Type 2 diabetic patients with nephropathy show structural-functional relationships that are similar to type 1 disease. *J Am Soc Nephrol*, 11(9), 1667–73.

White, K. E., Bilous, R. W., Marshall, S. M., *et al.* (2002). Podocyte number in normotensive type 1 diabetic patients with albuminuria. *Diabetes*, 51(10), 3083–9.

Wiegmann, T. B., Herron, K. G., Chonko, A. M., *et al.* (1992). Effect of angiotensin-converting enzyme inhibition on renal function and albuminuria in normotensive type I diabetic patients. *Diabetes*, 41(1), 62–7.

Williams, B., Shaw, A., Durrant, R., *et al.* (2005). Patient perspectives on multiple medications versus combined pills: a qualitative study. *QJM*, 98(12), 885–93.

Willich, S. N., Maclure, M., Mittleman, M., *et al.* (1993). Sudden cardiac death. Support for a role of triggering in causation. *Circulation*, 87(5), 1442–50.

Winer, N., Folker, A., Murphy, J. A., *et al.* (2005). Effect of fixed-dose ACE-inhibitor/calcium channel blocker combination therapy vs. ACE-inhibitor monotherapy on arterial compliance in hypertensive patients with type 2 diabetes. *Prevent Cardiol*, 8(2), 87–92.

Winkler, A. S., Marsden, J., Chaudhuri, K. R., *et al.* (1999). Erythropoietin depletion and anaemia in diabetes mellitus. *Diabet Med*, 16(10), 813–19.

Witte, D. R., Tesfaye, S., Chaturvedi, N., *et al.* (2005). Risk factors for cardiac autonomic neuropathy in type 1 diabetes mellitus. *Diabetologia*, 48(1), 164–71.

Wolf, G. and Ziyadeh, F. N. (2007). Cellular and molecular mechanisms of proteinuria in diabetic nephropathy. *Nephron Physiol*, 106(2), 26–31.
Wolf, G. and Ziyadeh, F. N. (1999). Molecular mechanisms of diabetic renal hypertrophy. *Kidney Int*, 56(2), 393–405.
Woolf, A. S., Gnudi, L., and Long, D. A. (2009). Roles of angiopoietins in kidney development and disease. *J Am Soc Nephrol*, 20(2), 239–44.
Wright, J. T., Jr., Bakris, G., Greene, T., *et al.* (2002). Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. *JAMA*, 288(19), 2421–31.
Writing Team for the Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications Research Group (2002). Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *JAMA*, 287(19), 2563–9.
Writing Team for the Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications Research Group (2003). Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy. *JAMA*, 290(16), 2159–67.
Yamamoto, T. and Ozono, K. (2001). [Role of advanced glycation endproducts in adynamic bone disease]. *Clin Calcium*, 11(8), 1044–7.
Yamamoto, Y., Maeshima, Y., Kitayama, H., *et al.* (2004). Tumstatin peptide, an inhibitor of angiogenesis, prevents glomerular hypertrophy in the early stage of diabetic nephropathy. *Diabetes*, 53(7), 1831–40.
Yanagisawa, M., Kurihara, H., Kimura, S., *et al.* (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, 332(6163), 411–15.
Yang, J., Zhang, D., Li, J., *et al.* (2009). Role of PPARgamma in renoprotection in type 2 diabetes: molecular mechanisms and therapeutic potential. *Clin Sci*, 116(1), 17–26.
Yang, J., Zhou, Y., and Guan, Y. (2012). PPARgamma as a therapeutic target in diabetic nephropathy and other renal diseases. *Curr Opin Nephrol Hypertens*, 21(1), 97–105.
Yasuda, G., Ando, D., Hirawa, N., *et al.* (2005). Effects of losartan and amlodipine on urinary albumin excretion and ambulatory blood pressure in hypertensive type 2 diabetic patients with overt nephropathy. *Diabetes Care*, 28(8), 1862–8.
Yip, J., Mattock, M. B., Morocutti, A., *et al.* (1993). Insulin resistance in insulin-dependent diabetic patients with microalbuminuria. *Lancet*, 342(8876), 883–7.
Yip, J. W., Jones, S. L., Wiseman, M. J., *et al.* (1996). Glomerular hyperfiltration in the prediction of nephropathy in IDDM: a 10-year follow-up study. *Diabetes*, 45(12), 1729–33.
Yusuf, S., Sleight, P., Pogue, J., *et al.* (2000). Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med*, 342(3), 145–53.
Zander, E., Schulz, B., Heinke, P., *et al.* (1989). Importance of cardiovascular autonomic dysfunction in IDDM subjects with diabetic nephropathy. *Diabetes Care*, 12(4), 259–64.
Zatz, R., Dunn, B. R., Meyer, T. W., *et al.* (1986). Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. *J Clin Invest*, 77, 1925.
Zatz, R., Meyer, T. W., Rennke, H. G., *et al.* (1985). Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. *Proc Natl Acad Sci U S A* 82(17), 5963–7.
Zerbini, G., Bonfanti, R., Meschi, F., *et al.* (2006). Persistent renal hypertrophy and faster decline of glomerular filtration rate precede the development of microalbuminuria in type 1 diabetes. *Diabetes*, 55(9), 2620–5.
Zhang, X., Lassila, M., Cooper, M. E., *et al.* (2004). Retinal expression of vascular endothelial growth factor is mediated by angiotensin type 1 and type 2 receptors. *Hypertension*, 43(2), 276–81.
Zhao, H. J., Wang, S., Cheng, H., *et al.* (2006). Endothelial nitric oxide synthase deficiency produces accelerated nephropathy in diabetic mice. *J Am Soc Nephrol*, 17(10), 2664–9.
Ziyadeh, F. N. (2004). Mediators of diabetic renal disease: the case for TGF-beta as the major mediator. *J Am Soc Nephrol*, 15 Suppl 1, S55–7.
Ziyadeh, F. N. and Han, D. C. (1997). Involvement of transforming growth factor-beta and its receptors in the pathogenesis of diabetic nephrology. *Kidney Int Suppl*, 60, S7–11.
Zoccali, C., Panuccio, V., Tripepi, G., *et al.* (2004). Leptin and biochemical markers of bone turnover in dialysis patients. *J Nephrol*, 17(2), 253–60.
Zoccali, C., Ruggenenti, P., Perna, A., *et al.* (2011). Phosphate may promote CKD progression and attenuate renoprotective effect of ACE inhibition. *J Am Soc Nephrol*, 22(10), 1923–30.
Zoja, C., Corna, D., Nava, V., *et al.* (2013). Analogues of bardoxolone methyl worsen diabetic nephropathy in rats with additional adverse effects. *Am J Physiol Renal Physiol*, 304(6), F808–19.
Zoja, C., Morigi, M., Figliuzzi, M., *et al.* (1995). Proximal tubular cell synthesis and secretion of endothelin-1 on challenge with albumin and other proteins. *Am J Kidney Dis*, 26(6), 934–41.
Zoja, C., Zanchi, C., Locatelli, M., *et al.* (2012). *FGF23 and Klotho Expression in the Kidney of Zucker Diabetic Fatty (ZDF) Rats and Modulation by ACE Inhibitor*. Presented at the American Society of Nephrology (ASN) Kidney Week 2012. <https://wwwasn-onlineorg/scripts/downloadaspx?file=/education/kidneyweek/archives/KW12Abstractspdf>

CHAPTER 150

Kidney involvement in plasma cell dyscrasias

Pierre M. Ronco

Overview

Monoclonal proliferations of the B-cell lineage are characterized by abnormal and uncontrolled expansion of a single clone of B cells at different maturation stages, with a variable degree of differentiation to immunoglobulin-secreting plasma cells. Therefore, they are usually associated with the production and secretion in blood of a monoclonal immunoglobulin and/or a fragment thereof which may become deposited in tissues. These deposits can take the form of casts (in myeloma cast nephropathy), crystals (in myeloma-associated Fanconi syndrome), fibrils (in light-chain and exceptional heavy-chain amyloidosis), or granular precipitates (in monoclonal immunoglobulin deposition disease) (Table 150.1). They may disrupt organ structure and function, inducing life-threatening complications. All of the pathologic entities related to immunoglobulin deposition principally involve the kidney, which is not only explained by the high levels of renal plasma flow and glomerular filtration rate, but also by the sieving properties of the glomerular capillary wall and by the prominent role of the renal tubule in light-chain handling and catabolism.

In a large proportion of patients with crystals, fibrils, or granular deposits of immunoglobulin products, major clinical manifestations and mortality are related to visceral immunoglobulin deposition rather than to expansion of the B-cell clone. Indeed, except for myeloma cast nephropathy, which is generally associated with a high tumour mass malignancy, immunoglobulin precipitation or deposition diseases often occur in the course of a benign B-cell proliferation and thus is consistent with monoclonal gammopathy of undetermined significance (MGUS) (Merlini and Stone, 2006; Leung et al., 2012). Since treatment is not recommended for MGUS, appropriate treatment such as chemotherapy and autologous stem cell autografting may be withheld, although preservation and even restoration of kidney function may be achieved upon successful treatment targeting the responsible clone. The International Kidney and Monoclonal Gammopathy Research Group proposed the term of monoclonal gammopathy of renal significance (MGRS) to stress the causal relationship between the monoclonal gammopathy and the renal disease, as well as the need for appropriate treatment (Leung et al., 2012).

The spectrum of renal diseases due to monoclonal immunoglobulin deposition has expanded dramatically with the advent of routine staining of renal biopsy specimens with specific anti-kappa and anti-lambda light-chain antibodies, and of electron microscopy, combined with more sensitive and sophisticated analyses of blood and urine monoclonal components (Table 150.2). Demonstration of MGRS primarily relies on immunohistochemical analysis of the kidney biopsy specimen because association of a circulating monoclonal component (M-component) with a renal disease does not prove causality and because detection of this component may be difficult in the blood and the urine. Owing to population ageing, the majority of patients with serum and/or urine monoclonal gammopathy who undergo renal biopsy have disease unrelated to monoclonal immunoglobulin deposition (Paueksakon et al., 2003). Therefore, the diagnosis of virtually all of the entities to be discussed in this chapter is critically dependent on the inclusion of kappa and lambda in the standard of immunofluorescence stains. A major consequence of such routine staining was the recent recognition of membranoproliferative glomerulonephritis secondary to intact monoclonal immunoglobulin deposition (Sethi and Fervenza, 2012) and proliferative glomerulonephritis with monoclonal immunoglobulin G (IgG) deposits mimicking immune-complex glomerulonephritis (Nasr et al., 2004, 2009). In some of the rarer entities, such as heavy-chain deposition disease (Moulin et al., 1999) and glomerulopathies with intact monoclonal immunoglobulin deposits, a more refined and precise diagnosis can be made with immunofluorescence staining for the subclasses of IgG with anti-heavy-chain antibodies. Collectively these stains may demonstrate light-chain isotype restriction and gamma-heavy-chain subclass restriction, which strongly favours, but does not definitely prove, the presence of a monoclonal immunoglobulin. Once such restriction has been identified, electron microscopy examination of the kidney specimen should be performed, particularly in patients with glomerulopathy, to look for organized structures in the kidney deposits (Table 150.2). In patients with amyloid deposits and inconclusive immunohistochemical analysis, identification of deposits may be achieved in specialized centres by immunoelectron microscopy (Veeramachaneni et al., 2004) and mass spectrometric-based proteomic analysis of deposits after laser microdissection of paraffin-embedded samples, a technique which will decrease the percentage of non-typable amyloidosis in the near future (Sethi et al., 2013).

Demonstration of monoclonality requires serum and urine studies by immunoelectrophoresis and immunofixation. Substantial advances have occurred with the immunonephelometric quantitation of serum free light chains. Although both kappa and lambda light-chain levels increase in patients with kidney failure, the ratio of free kappa to free lambda light chain remains altered in case of monoclonal immunoglobulin secretion. In AL amyloidosis, about 30% and 10% of patients will not have a demonstrable monoclonal

Table 150.1 Renal lesions observed in B-cell proliferations according to underlying haematological disease

Renal lesions	Multiple myeloma	Waldenström's macroglobulinaemia	Chronic lymphocytic leukaemia and related lymphomas
Tubular lesions			
Cast nephropathy	+ + +	–	–
(Proximal) tubule lesions[a]	+	–	–
Fanconi syndrome	+ (smouldering)	–	–
Glomerular lesions[b]			
AL amyloidosis	+ +	+	+
MIDD (nodular, membranoproliferative, minimal change)	+ +	+	–
Non-amyloid organized deposits[c]	–	–	+
Type I and type II cryoglobulinaemia	+	+ +	+ +
IgM capillary thrombi	–	+	–
Other (crescentic, minimal change, etc.)	+	+	+
Interstitial lesions			
B-cell infiltrate	+[d]	+ +	+ +
Nephrocalcinosis	+	–	–
Pyelonephritis (infections)	+	–	–

– = not or exceptionally observed; + to + + + = semiquantitative rating of the prevalence of renal lesions; MIDD = monoclonal immunoglobulin deposition disease.

[a] Without detectable myeloma casts, sometimes acute tubular necrosis.

[b] Glomerular involvement is usually but not always preponderant.

[c] Usually atypical membranous (or membranoproliferative) glomerulonephritis.

[d] Exceptionally, plasmacytoma.

light chain by immunoelectrophoresis and immunofixation, respectively, while the combination of immunochemical techniques and the serum free light-chain assay detects an abnormal result in 99% of patients (Palladini et al., 2009).

Polymorphism of renal lesions may be due to specific properties of immunoglobulin components influencing their precipitation, their interaction with renal tissue, or their processing after deposition. Alternatively, the type of renal lesions may be driven by the local response to immunoglobulin deposits, which may vary from one patient to another. That intrinsic properties of immunoglobulin components are responsible for the observed renal alterations was first suggested by *in vitro* biosynthesis of abnormal immunoglobulin by bone marrow cells from patients with lymphoplasmacytic disorders and visceral light-chain deposition (Preud'homme et al., 1980) and by recurrence of nephropathy in renal grafts (Leung et al., 2004). A further demonstration of the specificity of immunoglobulin component pathogenicity was provided by Solomon et al. (1991) who showed that the pattern of human renal lesions associated with the production of monoclonal light chains could be reproduced in mice injected with large amounts of light chains from the patients, which led to the conclusion that physicochemical or structural properties of light chains

Table 150.2 Pathologic classification of diseases featuring tissue deposition or precipitation of monoclonal immunoglobulin-related material

Organized			Non-organized	
Crystals	**Fibrillar**	**Microtubular**	**MIDD ('Randall type')**	**Other**
Myeloma cast nephropathy[a] Fanconi syndrome Other (extrarenal)	Amyloidosis (AL, AH) Non-amyloid	Cryoglobulinaemia kidney Immunotactoid	LCDD LHCDD HCDD	(Proliferative) GN with monoclonal IgG MPGN (IgG, IgA, or IgM)

AH = heavy-chain amyloidosis; AL = light-chain amyloidosis; GN = glomerulonephritis; HCDD, LCDD, LHCDD, MIDD = heavy-chain, light-chain, light- and heavy-chain, monoclonal immunoglobulin deposition disease; MPGN = membranoproliferative glomerulonephritis.

[a] Crystals are predominantly localized within casts in the lumen of distal tubules and collecting ducts, but may also occasionally be found in the cytoplasm of proximal tubule epithelial cells.

might be responsible for the specificity of renal lesions. A normal immunoglobulin is composed of two light chains and two heavy chains which are themselves made up of so-called constant (C) and variable (V) globular domains. Whereas a limited number of genes encode the constant region, multiple gene segments are rearranged to produce a variable domain unique to each chain. Diversity is further amplified by mutations and variations of the linking peptide segment. Although light chains and heavy chains have many structural similarities, they also possess a unique sequence that may be responsible for physicochemical peculiarities, hence their deposition in tissue or interaction with tissue constituents. A number of structural and physicochemical abnormalities of immunoglobulin have been described (reviewed in Solomon et al., 1982; Leboulleux et al., 1995). They include deletions of C_H domains in heavy-chain deposition disease (Aucouturier et al., 1993; Moulin et al., 1999) and heavy-chain amyloidosis (Eulitz et al., 1990), shortened or lengthened light chains and abnormal light-chain glycosylation in light-chain deposition disease (Preud'homme et al., 1980; Cogné et al., 1991), and resistance to proteolysis of the V_L fragment in Fanconi syndrome (Leboulleux et al., 1995). Moreover, overrepresentation of certain V_L gene subgroups was also reported in amyloidosis (Solomon et al., 1982; Ozaki et al., 1994) and light-chain deposition disease (Denoroy et al., 1994). It must be stressed, however, that some abnormal immunoglobulin chains produced in immunoproliferative disorders are not associated with any special clinical features. Conversely, structural abnormalities of light chains are not a constant feature of diseases associated with light-chain deposition. These observations suggest the need to increase the number of nephritogenic immunoglobulin components to be analysed at the complementary DNA and protein levels.

In the last decade, considerable progress has occurred in the treatment of patients with kidney involvement related to precipitation and deposition of monoclonal immunoglobulin or a fragment thereof. They are mostly due to a better control of the production and concentration of the M-component and to an improved monitoring of the patients. The combined administration of antiviral therapy and rituximab in patients with type II cryoglobulinaemia related to hepatitis C virus infection (see Chapter 151) and the development of new potent drugs such as the proteasome inhibitor bortezomib and thalidomide derivatives have substantially improved the outcome, particularly in those diseases such as AL amyloidosis and myeloma cast nephropathy where prognosis markedly depends on rapid control of the monoclonal proliferation (see Chapters 152 and 153). In parallel, new monitoring tools have appeared such as the free light-chain assay (Bradwell et al., 2003), and in patients with AL amyloidosis, the serum N-terminal pro-hormone brain natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponin T assays (Palladini et al., 2010). Ongoing trials of protein-leaking membranes will tell whether these membranes aimed at rapidly clearing circulating light chains will be of benefit in patients with severe renal failure related to myeloma cast nephropathy (Hutchison et al., 2007).

References

Aucouturier, P., Khamlichi, A. A., and Touchard, G. (1993). Brief report: heavy-chain deposition disease. *N Engl J Med*, 329, 1389–93.

Bradwell, A. R., Carr-Smith, H. D., Mead, G. P., *et al.* (2003). Serum test for assessment of patients with Bence Jones myeloma. *Lancet*, 361, 489–91.

Cogné, M., Preud'homme, J., Bauwens, M., *et al.* (1991). Structure of a monoclonal kappa chain of the V kappa IV subgroup in the kidney and plasma cells in light chain deposition disease. *J Clin Invest*, 87, 2186–90.

Denoroy, L., Déret, S., and Aucouturier, P. (1994). Overrepresentation of the V kappa IV subgroup in light chain deposition disease. *Immunol Lett*, 42, 63–6.

Eulitz, M., Weiss, D. T., and Solomon, A. (1990). Immunoglobulin heavy-chain-associated amyloidosis. *Proc Natl Acad Sci U SA*, 87, 6542–6.

Hutchison, C. A., Cockwell, P., Reid, S., *et al.* (2007). Efficient removal of immunoglobulin free light chains by hemodialysis for multiple myeloma: in vitro and in vivo studies. *J Am J Nephrol*, 18, 886–95.

Leboulleux, M., Lelongt, B., Mougenot, B., *et al.* (1995). Protease resistance and binding of Ig light chains in myeloma-associated tubulopathies. *Kidney Int*, 48, 72–9.

Leung, N., Bridoux, F., Hutchinson, C. A., *et al.* (2012). Monoclonal gammopathy of renal significance: when MGUS is no longer undetermined or insignificant. International Kidney and Monoclonal Gammopathy Research Group. *Blood*, 120, 4292–5.

Leung, N., Lager, D. J., Gertz, M. A., *et al.* (2004). Long-term outcome of renal transplantation in light-chain deposition disease. *Am J Kidney Dis*, 43, 147–53.

Merlini, G. and Stone, M. J. (2006). Dangerous small B-cell clones. *Blood*, 108, 2520–30.

Moulin, B., Deret, S., Mariette, X., *et al.* (1999). Nodular glomerulosclerosis with deposition of monoclonal immunoglobulin heavy chains lacking C(H)1. *Am J Soc Nephrol*, 10, 519–28.

Nasr, S. H., Markowitz, G. S., Stokes, M. B., *et al.* (2004). Proliferative glomerulonephritis with monoclonal IgG deposits: a distinct entity mimicking immune-complex glomerulonephritis. *Kidney Int*, 65, 85–96.

Nasr, S. H., Satoskar, A., Markowitz, G. S. *et al.* (2009). Proliferative glomerulonephritis with monoclonal IgG deposits. *Am J Soc Nephrol*, 20, 2055–64.

Ozaki, S., Abe, M., Wolfenbarger, D., *et al.* (1994). Preferential expression of human lambda-light-chain variable-region subgroups in multiple myeloma, AL amyloidosis, and Waldenström's macroglobulinemia. *Clin Immunol Immunopathol*, 71, 183–9.

Palladini, G., Barassi, A., Klersy, C., *et al.* (2010). The combination of high-sensitivity cardiac troponin T (hs-cTnT) at presentation and changes in N-terminal natriuretic peptide type B (NT-proBNP) after chemotherapy best predicts survival in AL amyloidosis. *Blood*, 116, 3426–30.

Palladini, G., Russo, P., Bosoni, T., *et al.* (2009). Identification of amyloidogenic light chains requires the combination of serum-free light chain assay with immunofixation of serum and urine. *Clin Chem*, 55, 499–504.

Paueksakon, P., Revelo, M. P., Horn, R. G., *et al.* (2003). Monoclonal gammopathy: significance and possible causality in renal disease. *Am J Kidney Dis*, 42, 87–95.

Preud'homme, J. L., Morel-Maroger, L., Brouet, J. C. *et al.* (1980). Synthesis of abnormal immnoglobulins in lymphoplasmacytic disorders with visceral light chain deposition. *Am J Med*, 69, 703–10.

Sethi, S. and Fervenza, F. C. (2012). Membranoproliferative glomerulonephritis-a new look at an old entity. *N Engl J Med*, 366, 1119–31.

Sethi, S., Theis, J. D., Vrana, J. A., *et al.* (2013). Laser microdissection and proteomic analysis of amyloidosis, cryoglobulinemic GN, fibrillary GN, and immunotactoid glomerulopathy. *Clin J Am Soc Nephrol*, 8(6), 915–21.

Solomon, A., Frangione, B., and Franklin, E. C. (1982). Bence Jones proteins and light chains of immunoglobulins. Preferential association of the V lambda VI subgroup of human light chains with amyloidosis AL (lambda). *J Clin Invest*, 70, 453–60.

Solomon, A., Weiss, D. T., and Kattine, A. A. (1991). Nephtotoxic potential of Bence Jones proteins. *N Engl J Med*, 324, 1845–51.

Veeramachaneni, R., Gu, X., and Herrera, G. A. (2004). Atypical amyloidosis: diagnostic challenges and the role of immunoelectron microscopy in diagnosis. *Ultrastruct Pathol*, 28, 75–82.

CHAPTER 151

The patient with cryoglobulinaemia

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Introduction

Cryoglobulinaemia is a pathological condition in which the blood contains immunoglobulins that have the property of reversible precipitation from human serum cooled to 4°C. The discovery in the human serum of proteins which reversibly precipitate in the cold was made by Wintrobe and Buell (1933). In 1947, Lerner and Watson found that these proteins were gammaglobulins and introduced the term cryoglobulins (cold precipitable serum globulin) (Lerner and Watson, 1947). A definite nosographic placement to the cryoglobulinaemic disease within the vast family of systemic vasculitis was made by Meltzer et al. (1966) who first described the clinical syndrome of essential mixed cryoglobulinaemia (MC), characterized by purpura, weakness, arthralgia, and in some patients, organ involvement (e.g. nephropathy and neuropathy). Histology typically reveals a leucocytoclastic vasculitis, with deposition of immunoglobulin (Ig)-M rheumatoid factor (RF), IgG, C3, and neutrophils in the small-sized vessel wall. A necrotizing vasculitis, with fibrinoid necrosis of the intima and the inflammation of the entire vessel wall and perivascular space, may also occur.

On the basis of immunochemical studies, Brouet et al. (1974) identified three types of cryoglobulins. In type I, the cryoprecipitable immunoglobulin is a single monoclonal immunoglobulin. Type II and III cryoglobulinaemias are both mixed types (MC), composed of at least two immunoglobulins. In both of them, a polyclonal IgG is bound to another immunoglobulin which is an antiglobulin and acts as a RF. The main difference between these two types of MCs is that in type II the RF usually of the IgM class, is monoclonal, whereas in type III it is polyclonal. Both components of MCs, IgG and IgM RF, are necessary for precipitations in the cold, whereas the individual components do not have this property (Fig. 151.1). Patients are considered to have a significant cryoglobulin level when it is > 0.05 g/L on two determinations. Some laboratories characterize cryoglobulinaemia using immunofixation or immunoelectrophoresis, and quantify the cryoglobulin level by determining the cryocrit, as the percentage of the total volume. The use of immunoblotting for immunochemical characterization is a sensitive and specific method allowing a full identification in 98% (Fabrizi et al., 2002). When a cryoglobulin is suspected, serum should be kept warm, and tests should be carried out at 37°C. Serum cryoglobulin may also interfere with a variety of laboratory tests and has been associated with spurious quantitation of plasma proteins and erythrocyte sedimentation rate, pseudoleucocytosis, pseudothrombocytosis or pseudomacrocytosis. Other laboratory abnormalities may provide surrogate evidence of the presence of cryoglobulinaemia such as low C4 serum complement fraction, decreased total haemolytic complement levels, presence of a serum monoclonal immunoglobulin, or RF activity.

MCs represent 60–75% of all cryoglobulinaemias, and are found in connective tissue diseases, and infectious or lymphoproliferative disorders, that is, secondary MC. Since the identification of hepatitis C virus (HCV) (Choo et al., 1989; Kuo et al., 1989) many authors have recognized it as the cause of > 80–90% of MC. HCV is primarily associated with type II MC (which typically has an IgMκ RF with anti-idiotypic activity), and to a lesser extent, with type III MC (Cacoub et al., 1999; Trejo et al., 2001; Saadoun et al., 2006a). In the absence of an identified aetiological factor (< 5% of all MC) cryoglobulinaemic vasculitis is defined as essential or idiopathic. This review aims to describe main characteristics of cryoglobulinaemia, HCV and non-HCV induced, with a special focus on kidney involvement.

Hepatitis C virus mixed cryoglobulinaemia and kidney disease

Epidemiology

Beside chronic liver disease, relevant extrahepatic manifestations of HCV infection include cryoglobulinaemia, lymphoproliferative disorders, and renal diseases. Several authors have given evidence of the association between HCV and glomerular disease in both native and transplanted kidneys (Cacoub et al., 2000; Cruzado et al., 2001). These manifestations of HCV are uncommon and the available information on their frequency is limited and mostly based on small studies without a control group. To date, the most important survey has been made by El-Serag et al. (2002) who carried out a hospital-based case–control study among US male veterans hospitalized between 1992 and 1999. They identified 34,204 patients who were hospitalized with HCV (cases) and 136,816 randomly selected patients without HCV (controls) who were hospitalized during the time period. According to their multivariate analysis, a significantly greater proportion of HCV-infected patients had porphyria cutanea tarda (0.77% vs 0.06%, $P < 0.0001$), vitiligo (0.17% vs 0.10%, $P = 0.0002$), lichen planus (0.30 vs 0.13, $P < 0.0001$), and cryoglobulinaemia (0.57% vs 0.05%, $P < 0.0001$). There was a greater proportion of membranoproliferative glomerulonephritis (MPGN) among patients with HCV (0.36% vs 0.05%, $P < 0.0001$), but not membranous glomerulopathy (0.33% vs 0.19%, $P = 0.86$).

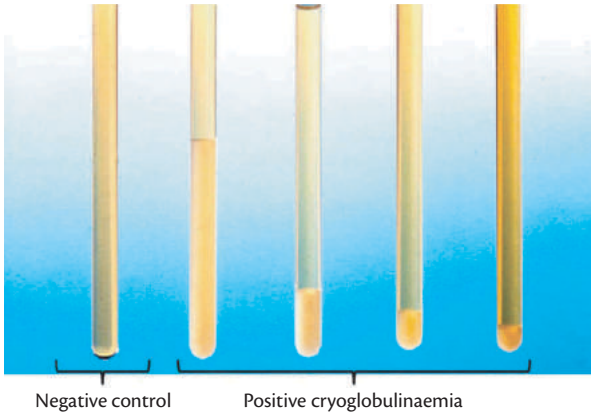

Fig. 151.1 Cryoprecipitation of circulating immunoglobulins in the serum of a patient with type II mixed cryoglobulinaemia. Cryoprecipitation can start within an hour or take several days.

During the last decade, some surveys extracted from large clinical databases have suggested an impact of HCV on prevalence and incidence of kidney disease in the general population. Patients with HCV might be more likely to have diabetes, obesity, or human immunodeficiency virus (HIV), these conditions being independently associated with kidney disease. Chronic HCV infection is associated with MC and MPGN, and these, in turn, can lead to kidney function impairment (Tsui et al., 2006; Dalrymple et al., 2007; Asrani et al., 2010; Lee et al., 2010; Butt et al., 2011). It remains unclear whether and to what extent HCV affects renal function (Table 151.1A). In contrast, four cross-sectional surveys have shown a significant link between HCV and proteinuria (Table 151.1B) (Lianpunsakul and Chalasani, 2005; Huang et al., 2006; Tsui et al., 2006; Lee et al., 2010). Multivariate logistic regression models showed that anti-HCV positive rate was significantly associated with proteinuria independently of common metabolic factors, such as diabetes mellitus, arterial hypertension, obesity, and dyslipidaemia (Huang et al., 2006). The possible linkage between HCV infection and proteinuria may be one part of HCV's extrahepatic manifestations. Type I MPGN associated with type II MC remains the most common form of kidney disease associated with HCV infection. Less frequently described kidney lesions are MPGN without cryoglobulinaemia, and membranous nephropathy. Occasional cases of focal segmental glomerulosclerosis, fibrillary or immunotactoid glomerulopathies, and thrombotic microangiopathy have been also reported (Fabrizi et al., 2002). In addition, vasculitis and interstitial nephritis have been associated with HCV. More recent information has been accumulated on the association between HCV infection and glomerular disease in liver- or kidney-transplanted populations (Montalbano et al., 2007). The natural history of these HCV-associated nephropathies is characterized by remission and relapsing phases; however, the long-term outcome is not well known.

Pathophysiology

Cryoglobulinaemia vasculitis is a systemic vasculitis, that mainly affects the small- and, less frequently, medium-sized arteries and veins. It is characterized by the deposition of immune complexes containing RF, IgG, HCV RNA, and complement on endothelial surfaces, eliciting vascular inflammation through poorly understood mechanisms. MC is also characterized by the proliferation of B-cell clones producing pathogenic IgM with RF activity. MC represents an example of immune complex vasculitis (Fig. 151.2). Intravascular cryoglobulin precipitation is induced by cold temperature and may involve primarily the skin, peripheral nerves, and kidney. A leucocytoclastic reaction is commonly involved in vessel damage of the cutaneous vasculitis, whereas in the peripheral nerve tissue of MC patients, T cells and macrophages are dominant infiltrating cells in vascular walls. In contrast to cutaneous vasculitis, HCV RNA has not been prominently detected in immune complexes in renal lesions, and has not been detected in all the peripheral neuropathy lesions. These findings suggest that different pathophysiological processes are involved in different target organs. The prevalent pathogenetic mechanism of HCV-associated cryoglobulinaemic glomerulonephritis is represented by the deposition in the glomerulus of a monoclonal IgM RF with particular affinity for the glomerular matrix, a RF produced by permanent clones of B lymphocytes infected by the virus. It is possible that in a minority of cases, immune complexes composed of HCV antigens and anti-HCV IgG antibodies deposit directly in the glomerular structures in the absence of a concomitant type II MC with a monoclonal IgM RF, indicating an immune complex glomerulonephritis similar to that observed in patients infected with hepatitis B virus. Such a mechanism could explain the non-cryoglobulinaemic GN rarely observed by others in patients with HCV chronic infection (Johnson et al., 1994).

Table 151.1 (A) Multivariate analysis for the association of hepatitis C to low estimated GFR: population-based surveys (B) Multivariate analysis for the association of anti-HCV seropositive rate to proteinuria: odds ratios (ORs) from population-based surveys

	OR (95% confidence intervals)	Country	Reference year
A			
Tsui et al.	0.89 (0.49–1.62)	US	2006
Tsui et al.	2.80 (2.43–3.23)	US	2007
Dalrymple et al.	1.40 (1.11–1.76)	US	2007
Lee et al.	1.30 (1.20–1.42)	Taiwan	2010
Asrani et al.	0.92 (0.79–1.08)	US	2010
Butt et al.	1.30 (1.23–1.37)	US	2011
B			
Liangpunsakul and Chalasani	1.99 (1.38–2.85)	US	2005
Huang et al.	1.648 (1.246–2.179)	US	2005
Tsui et al.	1.84 (1.0–3.37)	US	2006
Lee et al.	1.14 (1.003–1.30)	Taiwan	2010

The role of HCV itself and HCV-induced B-cell lymphoproliferation

Various pieces of evidence support the aetiological role of HCV in MC. A high prevalence of HCV RNA was detected in the great majority (up to 90%) of patients with type II essential MC (Agnello et al., 1992; Cacoub et al., 1994; Lunel et al., 1994; Fabrizi et al., 1998). An increased concentration (up to 10-fold) of IgG anti-HCV

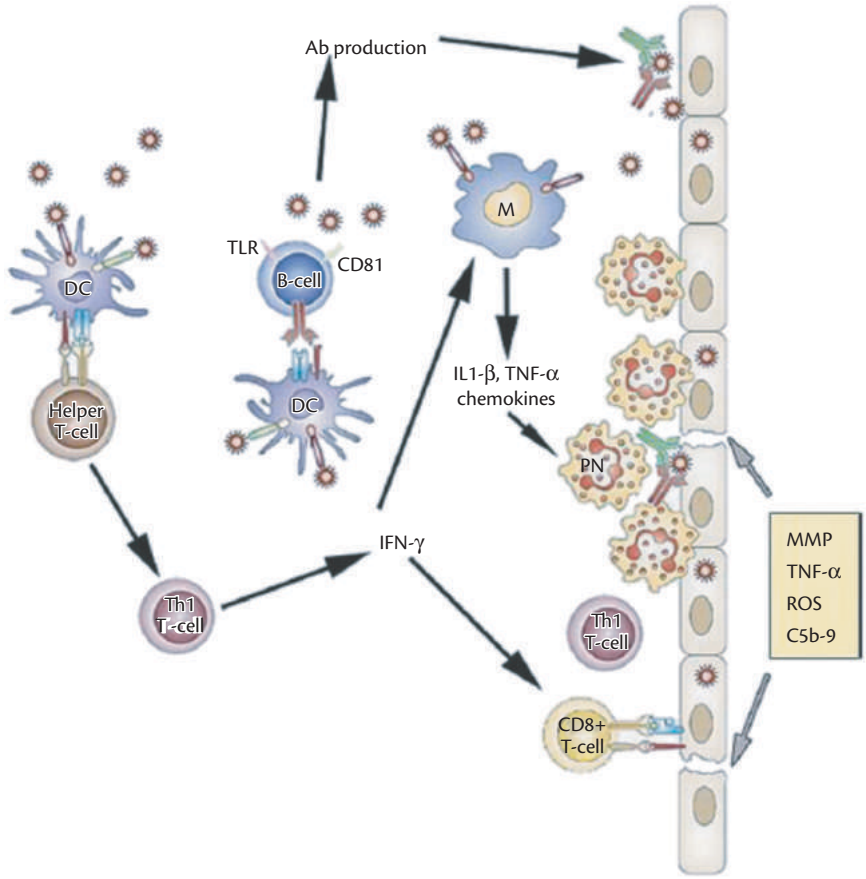

Fig. 151.2 Mechanisms responsible for HCV-MC-vasculitis lesions. HCV may infect B cells by CD81 and induce a chronic stimulation. B cells produce antibodies against HCV that crosslink with IgM with RF activity and may form immune complexes (i.e. cryoglobulin). Cryoglobulinaemia vasculitis is characterized by immune complex-mediated tissue injury linked to deposit of immune complexes and subsequent neutrophil recruitment and complement activation. Macrophages and T cells are also present in vasculitic tissues with a predominant Th1 type cytokine differentiation. The last step of vasculitis lesions involves matrix metalloprotease (MMP), oxidative stress molecules (ROS), and pro-inflammatory cytokines like TNF-α. DC = dendritic cells; M = macrophages; PN = polymorphonuclear neutrophil.

antibody in the cryoprecipitate was measured (Agnello et al., 1992). The majority of the known HCV antigens (core, E1, E2, NS34, NS4, and NS5) and their corresponding antibodies are described in both cryoprecipitate and vascular lesions in tissue sections (Sansonno et al., 1997). HCV RNA was found in the cryoprecipitate of patients with type II MC concentrated up to 1000 times the respective levels in supernatants (Agnello et al., 1992; Cacoub et al., 1994; Lunel et al., 1994).

HCV exerts a chronic stimulus on the immune system, which may lead to the proliferation of B-cell clones producing pathogenic IgM with RF activity. The basis for the strong association between this B-cell response to HCV and the detection of a RF in MC vasculitis may lie in the structural and antigenic homologies between the N-terminal region of the HCV E2 envelope protein and the human immunoglobulin variable domains. A possible contributory factor may be the ability of E2 envelope glycoprotein to bind CD81 B-cell surface protein which may provide a strong stimulatory signal if activated as a part of a complex (CD19/CD21/CD81 complex) together with BCR activation (Charles and Dustin, 2009). These findings strongly promote the use of antiviral agents and B-cell depletion therapy in the management of HCV-associated MC vasculitis. Charles and Dustin (2009) have suggested that specific HCV proteins are necessary for clonal B-cell expansion. High concentrations of HCV envelope protein E2 *in vitro* stimulate B-cell expansion via interaction with CD81, a known HCV E2 entry factor (Rosa et al., 2005). IgG-bound HCV specifically drives the clonal expansion of B cells secreting IgM RF; upon chronic HCV infection, immune-complexed HCV stimulates the expansion of V_H-1-69$^+$ B cells, encoding RF WA. These cells become clonally predominant by continued antigenic exposure (usually over a decade or more), independently of T-cell help. The HCV E2-CD81 interactions could result in a lowered B-cell stimulation threshold, facilitating the secretion of various antibodies, including IgM RF. Clonal B-cell expansions are demonstrable in the intrahepatic lymphocyte infiltrates, in the bone marrow, and in the peripheral blood mononuclear cells (Vallat et al., 2004; Charles et al., 2008). It is still not understood why such an expansion occurs more readily in chronic HCV infection, compared to other chronic viral diseases such as HBV or HIV. Increased serum B-cell activating factor (BAFF) (Sene et al., 2007), a tumour necrosis factor alpha (TNF-α) family member required for B-cell survival has been described in HCV-associated MC. It has been proposed that HCV rarely infects B cells, but HCV viral particles may be bound directly or indirectly to B cells. Marukian et al. (2008) have shown that B cells lack necessary HCV entry receptors and cannot support replication of HCV.

In a limited number of patients (< 10%), monoclonal B-cell expansion leading to type II MC may evolve into frank B-cell non-Hodgkin lymphoma. Transformation from polyclonal B-cell proliferation (type III MC) to oligo/monoclonal B cell proliferation (type II MC) and to the overt malignant lymphoma is a multistep process probably requiring multiple mutagenic events (Sene et al., 2004; Landau et al., 2007). The duration of B-cell stimulation caused by infectious or other exogenous agents has been also implicated. Recently, it has been shown the expansion of functionally anergic CD21-/low marginal zone-like B cell clones in HCV infection-related lymphoproliferation (Terrier et al., 2012).

The role of autoantibodies in cryoglobulinaemia vasculitis

The harmful role of cryoglobulins is supported by the presence of immunoglobulin molecules and complement fractions in the wall of affected microvessels free of cellular exudation. The complement system plays an active role. In a mouse model of cryoglobulin-induced immune complex glomerulonephritis, the neutrophil influx was shown to be mediated by C5 activation (Trendelenburg et al., 2005). Evidence also suggests that in MC, defective processing and decreased clearance of immune complexes favour their tissue deposition (Roccatello et al., 1993). MRL-*lpr*/*lpr* mice spontaneously develop a lupus-like syndrome characterized by necrotizing vascular lesions and severe glomerulonephritis. Skin and glomerular lesions similar to those associated with cryoglobulinaemic vasculitis can be induced in normal mice by injection of a monoclonal antibody exhibiting both cryoglobulin and RF activities derived from the MRL-*lpr*/*lpr* autoimmune mouse. Thus, both RF and cryoglobulin activities of the monoclonal antibody are required for the development of skin vasculitis, but its cryoglobulin activity alone is sufficient to cause glomerular lesions (Reininger et al., 1990).

Involvement of cellular immunity in cryoglobulinaemia vasculitis lesions

Cryoglobulinaemic vasculitis appears to be, at least in part, pathologically different from the Arthus model. Neutrophilic infiltration with leucocytoclastic changes, typical of immune complex-mediated vasculitis, has seldom been found, while the presence of lympho-histiocytic infiltrates suggests a T-cell mediated pathogenesis (Cacoub et al., 2001). Inflammatory infiltrates of lymphocytes and monocytes around small and pre-capillary arterioles is a feature of MC-vasculitis neuropathy. Monocytes and memory/activated T lymphocytes accounted for the bulk of leucocytic cells. Both CD4-positive and CD8-positive T cells accumulate in vasculitic nerves lesions. The role of cellular immunity and Th1 lymphocytes was demonstrated in the pathogenesis of HCV-related MC vasculitic nerve lesions (Saadoun et al., 2005).

Pathological features

The involvement of cryoglobulins in the context of clinical symptoms evocative of vasculitis may be confirmed with histological examination of the most frequently involved organs. Skin biopsy performed in patients with purpura and/or ulcers shows frequently a non-specific leucocytoclastic vasculitis involving small-sized vessels with inflammatory infiltrates and, in some cases, fibrinoid necrosis of the arteriolar walls and endovascular thrombi. In patients with peripheral neuropathy, nerve pathological analysis often highlights moderate to severe axonal damages (axonal degeneration, differential fascicular loss of axons, signs of demyelinization) associated with a small-sized vessel vasculitis (arterioles, venules, capillaries) and an inflammatory infiltrate composed only of monocytes and lymphocytes, without necrotizing angiitis (Fig. 151.3).

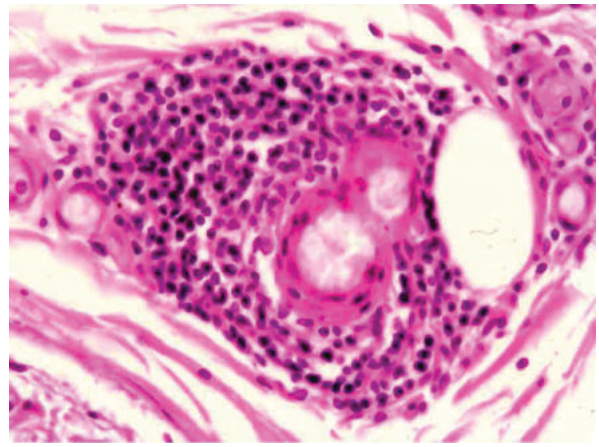

Fig. 151.3 Pathological features of cryoglobulinaemia vasculitis. Peripheral nerve biopsy: important peri-vascular infiltrate of lymphocyte around small-sized vessels, that is, venules, capillaries, and arterioles, with no polymorphonuclear infiltrate or destruction of the vascular wall.

Morphological features observed on kidney biopsies of patients with renal involvement are characterized by an important monocyte infiltrate with double contours of the basement membrane, large, eosinophilic, and amorphous intraluminal thrombi, evocative of MPGN. Immunofluorescence study shows intraglomerular subendothelial deposits of IgG, IgM (identical to those of the cryoprecipitates), and complement components. In addition, vasculitis of small renal arteries is present in one-third of patients. Extracapillary crescents are rarely observed (Beddhu et al., 2002).

Pathogenesis of HCV mixed cryoglobulinaemia kidney injury

Some evidence support that the kidney injury due to HCV infection is mediated by cryoglobulins. Cryoglobulins are deposited in the mesangium during their trafficking in the glomerulus. They can also be seen as intense subendothelial IgM deposits by immunofluorescence. Their nephrotoxicity is related to special affinity of the IgMκ RF for cellular fibronectin present in the mesangial matrix (D'Amico, 1998). It has been possible to induce, in an experimental mouse model, a MPGN similar to cryoglobulinaemic glomerulonephritis of humans by intravenous administration of 37°C solubilized type II cryoglobulins from patients with membranoproliferative nephritis and HCV (Fornasieri et al., 1993). The monoclonal IgMκ RF was isolated from such cryoglobulins, separately injected, and able to deposit in the glomerulus; this suggests a special affinity of IgMκ RF for the glomerular structures. It needs to be clarified if the deposition of a monoclonal IgM RF in the glomerulus occurs alone or as a mixed IgG–IgM cryoglobulin not bound to HCV or as a complex made of HCV, anti-HCV IgG, and IgMκ RF. Only the RF isolated from cryoprecipitable type II MC had specific affinity; all the other monoclonal RFs are not able to fix fibronectin.

The typical histopathological lesion is a membranoproliferative (mesangio-capillary) glomerulonephritis (Barsoum, 2007). Cryoglobulins can also be deposited in the glomerular capillaries as eosinophilic thrombi and this is usually associated with vasculitis and fibrinoid necrosis of the glomeruli. Endothelial injury may be an expression of the direct cytopathic activity of the virus. Cryoglobulins may also induce endothelitis via anti-endothelial antibody activity and complement activation leading to overexpression of VCAM-1 and subsequent platelet aggregation (Barsoum, 2007). Immune complexes containing HCV antigens have been observed in the mesangium of patients with cryoglobulinaemia leading to mesangial expansion (Sansonno et al., 2005). The presence of HCV-related proteins in the mesangium has been associated with higher proteinuria, possibly reflecting direct mesangial damage by HCV (Sansonno et al., 2005). An increased expression of toll-like receptors has been found in the mesangial cells target of HCV-related MPGN, but not non-HCV MPGN. Mesangial upregulation of toll-like receptors is linked with strong inflammatory activity (Wornle et al., 2006).

Clinical features

The clinical syndrome of MC vasculitis can be associated with both type II and III cryoglobulins. In the rheumatologic surveys, patients with type III MC outnumbered those with type II MC (Gorevic et al., 1980); conversely, surveys based on the description of renal involvement revealed a greater prevalence of type II MC, the monoclonal IgM component being mostly IgMκ (Tarantino et al., 1981). While in the few cases of type III MC with renal involvement the glomerular lesions were variable and non-specific, in type II MC, in which IgMκ was the monoclonal component, a specific well-characterized pattern of glomerular disease has been described, called 'cryoglobulinaemic glomerulonephritis' (D'Amico, 1998). Patients with MC usually show serum positivity for anti-HCV antibodies and HCV RNA in serum. Serum RF, which is elevated in 16–70% of HCV-positive patients, is usually increased in the setting of HCV-MC; the serum levels of C4 and C1q are usually very low. Some patients exhibit normal aspartate aminotransferase/alanine aminotransferase levels or only mild elevation of liver enzymes (60–70% of cases).

The amount of circulating cryoglobulin is usually measured as cryocrit, showing various levels in different patients and in the same patients at various times. The relationship between the severity of the extrarenal and renal manifestations and the cryocrit level is still discussed. The frequency of kidney involvement in MC varies from 8% to 58% of patients. In a minority of cases, the renal disease can be the first and unique presenting manifestation which makes the diagnosis of MC possible. More than half of patients have proteinuria and/or haematuria only. A nephritic syndrome is diagnosed in about 20% of cases. An acute nephritic syndrome, featured by haematuria, proteinuria, and a sudden rise in serum creatinine level develops in a similar proportion. Often both nephrotic and nephritic syndromes are simultaneously present. In 10% of patients, an acute oliguric kidney failure is the first indicator of kidney disease. Arterial hypertension is a frequent symptom, affecting > 50% of patients at the time of diagnosis. This complication is frequently severe and requires intense therapy. In many cases, a malignant hypertension is associated with rapidly progressive nephritis, while in others, refractory hypertension is independent of the severity of kidney disease (Tarantino et al., 1981). Signs of MC vasculitis usually precede the renal disease for many years; however, in 39% of cases, renal and extrarenal involvement are concurrent. Main extrarenal clinical features of MC include skin purpura, arthralgia, neuropathy, and hypocomplementaemia. Additional extrarenal signs are hepatomegaly, sicca syndrome, and central nervous system (CNS) and gut involvement (Saadoun et al., 2011b). Peripheral neuropathy has been mostly described as both a motor and sensory polyneuropathy, mainly distal, and of subacute onset, although cases of exclusively asymmetrical sensory neuropathy have been reported (Costa et al., 2003). Two main pathogenic mechanisms have been suggested, cryoglobulin deposition in the vasa vasorum, and necrotizing vasculitis. The first mechanism may account for the pattern of progressive sensory polyneuropathy and the second for the multineuropathy features (Authier et al., 1993; Saadoun et al., 2010). In some series, when electromyography was routinely made, an abnormal peripheral nerve conduction was observed in 70% of patients (Valli et al., 1989). Less frequently, patients may present with a CNS involvement due to cerebral vasculitis (Casato et al., 2005). Gastrointestinal manifestations are reported in 7.4% of patients with HCV-MC vasculitis. Abdominal pain, surgical abdomen, and/or intestinal bleeding were the main presentation. Patients with gastrointestinal manifestations showed more frequent renal (75% vs 30%; P = 0.003) and cardiac involvement (25% vs 2%; P = 0.006), and higher cryoglobulin levels (2.2 g/L vs 1.2 g/L; P = 0.07) (Terrier et al., 2010a). In many series, the pulmonary involvement was infrequent; however, when pulmonary function was routinely investigated, functional abnormalities related to the immunologic aggression to lung interstitium were seen in 61% of patients (Viegi et al., 1989).

The first clinical manifestations of type II MC usually appear in the fourth to fifth decade of life. Women outnumber men, and MC incidence varies in different geographical areas. The course of MC is usually characterized by periods of extrarenal symptoms alternated with periods of quiescence. The exacerbation of extrarenal symptoms is often associated with a flare of the renal disease, but it can occur independently. Renal disease in many patients shows an indolent course, and end-stage renal disease requiring dialysis is rare (< 10%); patients with cryoglobulinaemic nephritis have a poor prognosis mainly because of a high incidence of infectious and cardiovascular diseases (Terrier et al., 2011b).

A very large series was made by Roccatello et al. (2007), who included 146 patients with cryoglobulinaemic nephritis, of whom 87% (N = 127) were HCV positive. Type II cryoglobulin (IgG/IgMκ) occurred in 74.4% of cases. The remainder had type III cryoglobulins. A diffuse MPGN was the most common histologic pattern (83%). A multivariate Cox regression proportional hazard model showed that age, serum creatinine level, and proteinuria at onset of renal disease were associated independently with risk for developing severe renal failure at follow-up. Survival at 10 years was about 30% and cardiovascular disease was the cause of death in > 60% of patients; additional causes of death included infections (10%), hepatic failure (19%), and neoplasia (3%). Kaplan–Meier survival curves were worsened by baseline serum creatinine > 1.5 mg/dL. These findings are greatly different from those observed by Tarantino et al. (1995) who enrolled 105 patients and showed that the number of deaths caused by infections (21%) and hepatic failure (19%) approached the number of deaths caused by cardiovascular diseases (29%). These conflicting results have been attributed to different use of antibiotics, antiviral agents, or immunosuppressive drugs. In a recent study

of 151 consecutive HCV RNA-positive patients with MC vasculitis prospectively followed up between 1993 and 2009, baseline factors associated with a poor prognosis were the presence of severe liver fibrosis (hazard ratio (HR), 5.31), CNS involvement (HR 2.74), kidney involvement (HR 1.91), and heart involvement (HR 4.2). Use of antiviral agents was associated with a good prognosis, whereas treatment with immunosuppressant agents had a negative impact. The 1-year, 3-year, 5-year, and 10-year survival rates were 96%, 86%, 75%, and 63%, respectively (Terrier et al., 2011b).

Therapeutic management of HCV-associated mixed cryoglobulinaemia

The discovery of HCV and the analysis of pathophysiological mechanisms provided the opportunity to control HCV-MC with: (a) antiviral therapy based on the belief that the underlying infection is driving immune complex formation and resultant vasculitis, (b) B-cell depletion therapy targeting B cells which produce cryoglobulin, and (c) non-specific immunosuppressive therapy targeting inflammatory cells present in vasculitic lesions. Potential adverse effects of immunosuppressive therapy with glucocorticoids and cytotoxic drugs on an underlying chronic viral infection are a matter of concern.

Antiviral agents

The treatment of HCV infection (i.e. in the absence of HCV-MC) has progressed dramatically over the past 20 years with the standard of pegylated interferon alpha (PEG-IFN-α) and ribavirin therapy leading to sustained virological clearance in nearly half of patients. The early attempts to control HCV-MC with standard thrice weekly IFN-α was not surprisingly associated with a relatively poor response and a high relapse rate because of its low antiviral efficacy, especially in severe cases (Casato et al., 1997). IFN-α monotherapy was effective in 50–100% of patients with purpuric skin lesions, but did not demonstrate efficacy on neurological or renal involvement. Clinical improvement of HCV-MC correlated with virological response and when follow-up was sufficient, most of the responders developed virological and clinical relapses following IFN-α withdrawal (Dammacco et al., 1994; Misiani et al., 1994; Casato et al., 1997). In three uncontrolled studies (Zuckerman et al., 2000; Naarendorp et al., 2001; Cacoub et al., 2002), combination therapy with standard IFN-α and ribavirin demonstrated enhanced efficacy on main HCV-MC manifestations (cutaneous, 100%; renal, 50%; and neurological, 25–75%). Two studies reported a loss of proteinuria and haematuria in sustained virological responders without significant changes in renal function, regardless of the virological response to treatment (Bruchfeld et al., 2003; Alric et al., 2004). The better antiviral regimen with PEG-IFN-α plus ribavirin was used in 72 consecutive HCV-MC patients (Saadoun et al., 2006a) and permitted to achieve a higher rate of complete clinical (67.5% vs 56.2%) and virological response (62.5% vs 53.1%) as compared with standard IFN-α plus ribavirin, regardless of HCV genotype and viral load. In multivariate analysis, an early virological response at month 3 (odds ratio (OR), 3.53) was independently associated with a complete clinical response of MC whereas a glomerular filtration rate (GFR) < 70 mL/min (OR 0.18) was negatively associated with a complete clinical response.

A recent meta-analysis of controlled clinical trials comparing the efficacy and safety of antiviral versus immunosuppressive therapy (corticosteroids alone or with cyclophosphamide) in patients with HCV-induced glomerulonephritis showed that proteinuria decreased more (OR 3.86) after antiviral therapy (IFN-α monotherapy for at least 6 months). However, both treatments failed to significantly improve renal dysfunction. Of note, in all patients with proteinuria reduction, an HCV RNA clearance was shown at the end of antiviral therapy (Fabrizi et al., 2007).

Although 60–70% of HCV-MC patients exhibit sustained virological response with PEG-IFN-α plus ribavirin combination for 13–14 months, 30–40% of such patients remain refractory to this antiviral therapy. Specifically targeted antiviral therapy for HCV (STAT-C) might represent promising drugs to increase the virological response rate and thus, the clinical and immunological response of MC vasculitis (Hezode et al., 2009; McHutchison et al., 2009). In an open-label, prospective, single-centre cohort study (Saadoun et al., 2014), the efficacy of an NS3 protease inhibitor (boceprevir or telaprevir) in combination with PEG-INF-α2a (180 micrograms) or 2b (1.5 micrograms/kg) and ribavirin (800–1400 mg/day) was evaluated in 13 HCV-cryoglobulinaemia vasculitis patients. The main HCV-cryoglobulinaemia vasculitis manifestations included purpura (N = 10), polyneuropathy (N = 10), arthralgia (N = 6), and kidney involvement (N = 3). After 1 month of the PEG-IFN-α/ribavirin/protease inhibitor combination, 11 patients (85%) showed an early virological response (HCV-RNA level <1.1 log copies/mL). Nine patients showed a complete clinical response of cryoglobulinaemia vasculitis and four were partial responders. After 3 months of PEG-IFN-α/ribavirin/protease inhibitor combination therapy, the MC serum level dropped from 1.3 to 0.3 g/L, while the C4 serum level increased from 0.09 to 0.13 g/L. However, such combination therapy should be given for 48 weeks and serious adverse events occurred in up to 47% of patients. Other direct-acting antivirals are now becoming available. The NS3/4A inhibitor simeprevir and NS5B inhibitor sofosbuvir have recently been licensed. These agents facilitate the use of shortened courses of combination IFN-free therapy, which are associated with high (> 95%) SVR rates and relatively few toxicities. International guidelines (i.e. European Association for the Study of the Liver 2014 guidelines) state that treatment should be scheduled, not deferred, for patients with significant liver fibrosis and those with significant extrahepatic manifestations, such as symptomatic cryoglobulinemia. As far as SVR has been previously associated to mixed cryoglobulinemia vasculitis remission in most patients, there is no doubt that new HCV treatments should add a major benefit.

B-cell depletion therapy

Several Italian groups have reported on the efficacy of anti-CD20 monoclonal antibody, rituximab (RTX), in patients with HCV-MC vasculitis resistant or intolerant to IFN-α monotherapy (Sansonno et al., 2003; Roccatello et al., 2004). A complete clinical response was reported in 60–70% of cases, with cryoglobulin clearance in one-third of patients. However, the absence of efficacy on HCV clearance and the high relapse rates supported the need for combined antiviral therapy to block the HCV infection trigger and obviate long-term liver complications (Cacoub et al., 2008). A recent prospective controlled trial showed the superiority of a combination of RTX plus PEG-IFN α2b-ribavirin compared to PEG-IFN-α2b-ribavirin in severe HCV-associated MC vasculitis, with a more rapid improvement, a higher rate of kidney complete response, and a good safety profile (Saadoun et al., 2010). RTX plus PEG-IFN-α2b-ribavirin induced a clinical complete (CR) and partial (PR) response in 80%

and 15% of cases, respectively; an immunological CR and PR in 67% and 33%, respectively; and a sustained virological response in 55%. A similar superiority of the RTX plus PEG-IFN-α-ribavirin regimen has been reported by another group (Dammacco et al., 2010).

The efficacy and tolerance of RTX, with and without combined antiviral therapy, in a large cohort of HCV-vasculitis patients with a long-term follow-up was recently reported (Terrier et al., 2009). RTX alone induced a clinical CR and PR in 58% and 9% of patients, and immunological CR and PR in 46% and 36%, respectively. Patients treated with RTX without antiviral therapy showed stable levels of HCV RNA and a slight non-significant increase of alanine aminotransferase levels (1.5 times the upper limit of normal value at baseline to 1.7 times at the end of follow-up). These findings are reassuring regarding the use of RTX in HCV-infected patients, even in the absence of antiviral therapy. However, in rare cases, RTX may form a complex with RF-positive IgMκ, leading to accelerated cryoprecipitation and to severe systemic reactions. To decrease the risk of such side effect, RTX should be administered with the use of 375 mg/m^2/week protocol (4 consecutive weeks) and sometimes plasma exchanges prior to RTX infusion in patients with high baseline values of MC (Sene et al., 2009).

Immunomodulation

Patients with HCV cryoglobulinaemic vasculitis have been shown to have a reversible quantitative defect of the CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells (Tregs) after resolution of HCV infection and vasculitis cure (Landau et al., 2008). Interleukin 2 (IL-2), a cytokine that promotes Treg survival and function, could be beneficial for such patients who are resistant to HCV therapy. The safety and immunological effects of low-dose IL-2 were recently reported in a prospective, open-label phase I/IIa study (Saadoun et al., 2011a). Ten patients with HCV-cryoglobulinaemia vasculitis that was refractory to conventional antiviral and/or RTX therapy received one IL-2 course of 1.5 million IU/day for 5 days, followed by three 5-day courses of 3 million IU/day at weeks 3, 6, and 9. There were no drug-related adverse events greater than grade 1. The treatment did not induce effector T-cell activation, vasculitis flare, or increased viraemia. Improvement of the vasculitis symptoms was found in 8 of 10 patients. Administration of low-dose IL-2 was followed by an increase in the percentage of CD4$^+$CD25^{hi}CD127$^-$Foxp3$^+$ Tregs, with potent suppressive activity in all subjects, and a concomitantly decreased proportion of marginal zone B cells.

Non-specific immunosuppressive agents

Immunosuppressive agents have been given to MC patients with severe disease manifestations such as MPGN, severe neuropathy, and life-threatening complication. A combination of corticosteroids and immunosuppressant such as cyclophosphamide and azathioprine has been used while awaiting the generally slow response to antiviral treatments. In a large retrospective study of 105 patients with MC-associated renal disease associated with cryoglobulinaemia vasculitis, 80% were administered corticosteroids and/or cytotoxic agents, while 67% underwent plasmapheresis (Tarantino et al., 1995). Despite this aggressive approach, long-lasting remission of the renal disease was achieved in only 14% of cases, and the 10-year survival rate was only 49%.

Corticosteroids, used alone or in addition to IFN-α, did not favourably affect the response of HCV-related vasculitis manifestations in two controlled studies (Misiani et al., 1994; Casato et al., 1997). In one randomized trial, methylprednisolone alone given for 1 year was associated with clinical response in 22% of patients, compared with 66% and 71% in patients receiving IFN-α or IFN-α plus methylprednisolone, respectively (Dammacco et al., 1994). Low-dose corticosteroids may help to control minor intermittent inflammatory signs such as arthralgia but do not succeed in cases of major organ involvement (i.e. neurological, renal), or in the long-term control of MC vasculitis.

Plasmapheresis offers the theoretical advantage of removing the pathogenic cryoglobulins from the circulation in order to avoid the rebound increase in cryoglobulinaemia commonly seen after discontinuation of apheresis. When used in combination with anti-HCV treatment, plasmapheresis did not modify the virological response if IFN-α was given after each plasma exchange session (Hausfater et al., 2002).

Therapeutic guidelines

It appears logical that aggressive antiviral therapy with PEG-IFN and ribavirin be considered as induction therapy for HCV-MC with mild to moderate disease severity and activity (i.e. without rapidly progressive nephritis, motor neuropathy, or other life-threatening complications) (Fig. 151.4). The duration of therapy has not yet been rigorously determined but treatment courses longer than those merely based on genotype alone appear more likely to be effective, that is, at least 13 months (Cacoub et al., 2002). Of note, optimal antiviral regimen will be probably soon modified with new anti-HCV drugs (such as protease inhibitors) to increase the rates of sustained virological response. In patients presenting with severe disease (i.e. progressive renal disease, progressive motor neuropathy, extensive skin disease including ulcers and distal necrosis), an induction phase with RTX and/or plasmapheresis may be useful, before starting HCV treatment.

Non-HCV mixed cryoglobulinaemic vasculitis

Epidemiology

The prevalence and the incidence of cryoglobulinaemic vasculitis are unknown, in particular because of the heterogeneity in the cause, the clinical presentation, and the geographical distribution. Before the HCV era, the prevalence of the disease was reported as approximately 1:100,000 individuals (Gorevic et al., 1980). Idiopathic cryoglobulinaemic vasculitis was suggested to be more common in Southern Europe than in Northern Europe or North America. This finding was mainly related to the higher prevalence of HCV infection in such countries, when studies included HCV-positive and -negative patients (Cacoub et al., 1994; Ferri et al., 2002). In contrast to HCV infection, idiopathic cryoglobulinaemia vasculitis is considered to be a rare disorder, but no recent study has evaluated the prevalence of the disease. Idiopathic cryoglobulinaemia vasculitis appears more common in patients aged 45–65 years, with a maximum incidence in women (sex ratio of women:men is 2–3:1) (Ferri et al., 2002; Saadoun et al., 2006b). No predominant ethnicity is found.

Cryoglobulinaemic vasculitis can cause significant morbidity and mortality. In the literature, the worse prognostic factors were age (> 60 years) and renal involvement (Tarantino et al., 1995; Ferri

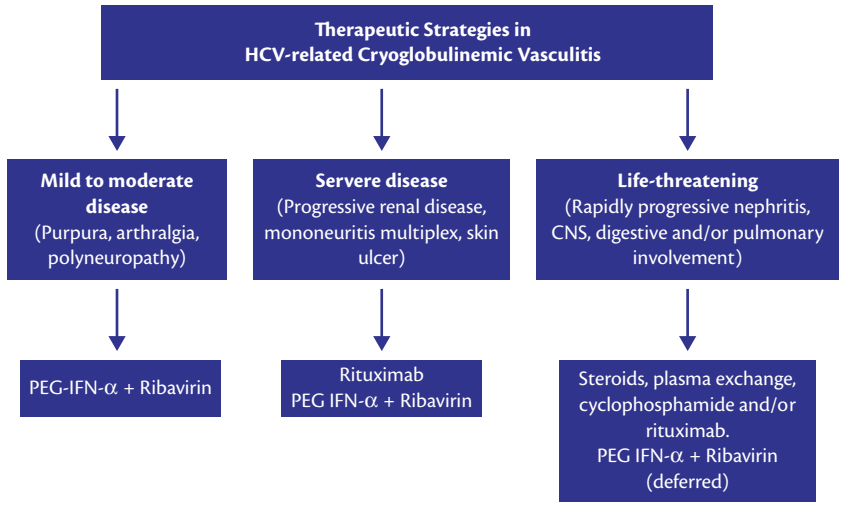

Fig. 151.4 Treatment of HCV-related mixed cryoglobulinaemia vasculitis according to the clinic-biological presentation.

et al., 2004). Renal involvement was reported as the main cause of death (Meltzer et al., 1966; Gorevic et al., 1980; Tarantino et al., 1995; Ferri et al., 2004; Della Rossa et al., 2010), followed by liver involvement, cardiovascular disease, infection, and lymphoma (Ferri et al., 2004). However, as indicated previously, most of these results were derived from old heterogeneous studies, including mostly HCV-positive and -negative MC patients, which may differ in terms of therapeutic management and outcome, in particular regarding liver complications. A retrospective study in the HCV era, including non-HCV patients with MC, reported a poor outcome and a fourfold increased risk of developing B-cell non Hodgkin lymphoma (B-NHL). In multivariate analysis, a serum cryoglobulin level > 0.6 g/L (OR 1.44) and the presence of cryoglobulinaemia vasculitis (OR 4.3) and hypogammaglobulinaemia (OR 6.7) were independently associated with B-NHL. Fourteen per cent of patients had died, primarily of serious infections. An age at diagnosis > 60 years (OR 1.06) and renal involvement (OR 5.20) were independently associated with death (Ramos-Casals et al., 2006; Saadoun et al., 2006b).

Clinical features

The most frequently targeted organs of non-HCV-MC vasculitis are skin, joints, nerves, and kidney. The disease expression is variable, ranging from mild clinical symptoms (purpura, arthralgia) to fulminant life-threatening complications (glomerulonephritis, widespread vasculitis) (Gorevic et al., 1980; Invernizzi et al., 1984; Monti et al., 1995; Ferri et al., 2002). The rates of most clinical and immunological manifestations of MC vasculitis are quite similar in HCV-positive and -negative patients (Table 151.2) (Trejo et al., 2001; Saadoun et al., 2006b; Ferri, 2008). However, renal manifestations are more frequently reported in HCV-negative patients, in 14–63 % of patients (Trejo et al., 2001; Saadoun et al., 2006b; Ferri et al., 2008). The most frequent clinical and histological picture is an acute or chronic type I MPGN with subendothelial deposits, representing 70–80% of MC renal diseases, which is strongly associated with the type II IgMκ MC (D'Amico 1998; Beddhu et al., 2002). The most frequent presentation is a proteinuria with microscopic haematuria and a variable degree of renal insufficiency (Tarantino et al., 1995). Nephrotic proteinuria or acute nephritic syndrome can also reveal MC renal involvement. New-onset arterial hypertension is seen in 80% of cases.

A French retrospective series of 20 patients with non-HCV-MC and renal disease showed that MC was related to primary Sjögren syndrome in nine patients and non-Hodgkin lymphoma in one patient, while it was classified as essential in the remaining 10 cases. Renal involvement was characterized by microscopic haematuria in all patients, nephrotic range proteinuria in 75% of patients, hypertension in 80% of patients, and renal failure in 85% (mean GFR 46 mL/min/1.73 m^2). MPGN with subendothelial deposits was observed in all kidney specimens. Skin vasculitis was the main extrarenal manifestation. In all patients, cryoglobulinaemia was classified as type II MC (Matignon et al., 2009).

Interestingly, a recent study analysed baseline factors associated with prognosis in patients with HCV-negative cryoglobulinaemic vasculitis (Terrier et al., 2013a). It found results that were quite similar to those in HCV-positive cryoglobulinaemic vasculitis, as the 1-year, 2-year, 5-year, and 10-year overall survival rates were 91%, 89%, 79%, and 65%, respectively. Deaths were related to serious infections in half the cases and to vasculitis flare in 20%.

Aetiology

The proportion of aetiological factors in two European studies is detailed in Table 151.3 (Trejo et al., 2001; Saadoun et al., 2006a). Type I cryoglobulins are always linked to a B-cell lymphoproliferative disorder. Type II and III MCs may be linked to B-cell lymphoproliferative disorder, autoimmune disorders, and/or infections (Saadoun et al., 2006a). As indicated previously, HCV infection is the most frequent cause of MC, representing 70–80% of cryoglobulinaemic vasculitis cases (Cacoub et al., 1999; Trejo et al., 2001; Saadoun et al., 2006b). Non-HCV-MC represents 10–25% of cases.

In our experience, type II MC are more frequently symptomatic than type III MC, with more frequent purpura, renal involvement, low C4 complement level, and higher cryoglobulin level (Saadoun et al., 2006a). Other groups found comparable clinical presentation between type II and type III MC vasculitis (Ferri et al., 2002). Type II MC are more frequently associated with haematological disorders than type III MC. Patients without HCV infection are more often women with more frequent renal involvement, B-NHL,

Table 151.2 Main clinical and immunological findings in HCV-positive and negative patients with cryoglobulinaemic vasculitis

Findings	Trejo et al., 2001	Saadoun et al., 2006b	Saadoun et al., 2006b	Ferri, 2008
N	206	118	65	250
HCV status	HCV positive and negative	HCV positive	HCV negative	HCV positive (92%) and negative
Clinical features				
Age at disease onset, mean ± SD years (range)	–	65 ± 14	64 ± 15	54 ± 13
Female/male ratio	–	1.1	2.2	3
Purpura	54%	59%	49%	98%
Weakness	22%	–	–	98%
Arthralgias	44%	45%	58%	91%
Arthritis (non-erosive)	–	–	–	8%
Sicca syndrome	–	–	–	51%
Peripheral neuropathy	17%	43%	31%	81%
Renal involvement	56%	14%	63%	31%
B-NHL	–	14%	35%	11%
Laboratory features				
Cryocrit, mean ± SD	–	0.9 ± 0.9 g/L	1.2 ± 1.6 g/L	4.4 ± 12%
Type II/type III mixed cryoglobulins	–	5.6/1	5.5/1	2/1
C3, mean ± SD mg/dL (normal 60–130)	–	–	–	93 ± 30
C4, mean ± SD mg/dL (normal 20–55)	–	–	–	10 ± 12

Table 151.3 Proportion of main aetiological factors in patients with cryoglobulinaemia

Aetiological factors	Trejo et al., 2001	Saadoun, 2006b
N	443	1434
Infection	75%	92%
Hepatitis C virus	73%	91%
Hepatitis B virus	3%	–
HIV	19%	–
Autoimmune disorders	24%	3%
Primary Sjögren syndrome	9%	–
Systemic lupus erythematosus	7%	–
Rheumatoid arthritis	0.5%	–
Haematological disorders	7%	2.5%
Non-Hodgkin lymphoma	4%	–
Chronic lymphocytic leukaemia	1%	–
Multiple myeloma	1%	–
Hodgkin lymphoma	0.5%	–
Essential or idiopathic cryoglobulinaemia	11%	2.5%

low C4 complement level, hypogammaglobulinaemia and high cryoglobulin level, but less frequent RF activity than HCV-infected patients (Saadoun et al., 2006a).

Treatment of cryoglobulinaemia vasculitis in patients without HCV infection

In contrast to HCV-MC, the therapeutic management of non-HCV-MC vasculitis has yet to be defined since no study has evaluated the best strategies. In patients with mild to moderate cryoglobulinaemia vasculitis, treatment may include the avoiding of cold temperatures, resting in case of purpura, and non-aggressive medications such as non-steroidal anti-inflammatory drugs, colchicine, and dapsone. Treatment of severe cryoglobulinaemia vasculitis is based on a combination of corticosteroids and immunosuppressants or plasmapheresis, sometimes associated with iloprost, low-dose aspirin, and/or anticoagulant in case of distal ischaemia. However, the results of these treatments are often disappointing (Tarantino et al., 1995). Besides the targeting of inflammatory cells with non-specific immunosuppressive therapy, the opportunity to target cryoglobulin-producing B cells with specific agents such as RTX has emerged.

Non-specific immunosuppressive agents are typically given to patients with severe disease manifestations such as MPGN, severe neuropathy, and other life-threatening complications. A combination of corticosteroids and immunosuppressant such as cyclophosphamide, chloraminophene, and azathioprine has been used. The benefit of plasmapheresis is controversial with the risk of relapse or rebound at discontinuation.

B-cell depletion therapy has been recently used in patients with cryoglobulinaemic vasculitis. Data on the efficacy and safety of RTX in non-viral cryoglobulinaemia vasculitis are scarce, since only a few case reports have been reported in the literature (Arzoo et al., 2002; Zaja et al., 2003; Koukoulaki et al., 2005; Nehme-Schuster et al., 2005; Basse et al., 2006; Bryce et al., 2006; Cohen et al., 2007; Braun et al., 2008; Pandrangi et al., 2008; Quartuccio et al., 2008; Cavallo et al., 2009; Ruch et al., 2009; Annear et al., 2010). A French study recently reported on the safety and efficacy of RTX in the largest series of 23 patients with non-viral cryoglobulinaemic vasculitis (Terrier et al., 2010a). RTX showed a clinical and immunological response in > 80%. However, side effects were noted in almost half of patients, including severe infections in 26%, with a rate of 14.1/100 patient-years. These infections occurred in patients with age > 70 years, essential type II MC, renal failure with GFR < 60 mL/min, and receiving high-dose corticosteroids. Interestingly, long-term follow-up of non-viral cryoglobulinaemic vasculitis patients before the RTX era also revealed an increased risk of death, due primarily to sepsis, in patients > 60 years with renal involvement (Saadoun et al., 2006a). Similar results have been recently reported from a

large cohort of 242 patients presenting with non-infectious MC vasculitis (Terrier et al., 2012). These findings emphasize the intrinsic poor prognosis of this disorder and do not allow conclusions to be drawn about a specific toxicity attributable to RTX.

Type I cryoglobulinaemia

Type I cryoglobulins are always linked to a B-cell lymphoproliferative disorder, that is, multiple myeloma, Waldenström macroglobulinaemia, chronic lymphocytic leukaemia, B-NHL, and hairy cell leukaemia. Type I cryoglobulinaemic vasculitis presentation is often severe, in part because of high cryoglobulin levels, with frequent cutaneous and renal involvement (Monti et al., 1995; Trejo et al., 2001). Renal involvement has been described rarely in type I cryoglobulinaemia. In their first description, Brouet et al. (1974) reported that 25% of all cases of type I cryoglobulinaemia developed kidney disease but no description of histologic lesions was made. A more recent study reported that renal involvement occurred in around 40% of patients with type I cryoglobulinaemia (Trejo et al., 2001). By contrast, only 30 cases of type I cryoglobulinaemia with biopsy-proven glomerulonephritis have been reported in the medical literature to date (Ponticelli et al., 1977; Grcevska et al., 1998; Karras et al., 2002). Some of these cases resulted from a monoclonal IgM component, but the majority was related to IgG monoclonal component (with κ light chain in the majority of patients studied). When explored, hepatitis C serology was always negative.

Kidney disease was essentially a nephrotic syndrome with microscopic haematuria and renal failure (Karras et al., 2002). Serum creatinine ranged between 0.94 and 9.42 mg/dL. Serum component was low in most of the patients (10/17 = 59%), and RF activity was present in approximately half of them (9/17 = 53%). Renal histology showed almost constantly MPGN with monotypic immunoglobulin deposits corresponding to the monoclonal serum cryoglobulin. Capillary obstruction and interstitial mononuclear cell infiltration was also commonly noted. Electron microscopy frequently revealed fibrillary or microtubular deposits. Partial remission was achieved in some cases, especially when patients were treated with cyclophosphamide or chlorambucil associated with corticosteroids.

In a very recent French nationwide survey, 64 patients with type I cryoglobulinaemic vasculitis have been reported including 28 patients with monoclonal gammopathy of unknown significance (MGUS) and 36 with haematological malignancy (Terrier et al., 2013b). Type I cryoglobulinaemic vasculitis was characterized by severe cutaneous involvement (necrosis and ulcers) in almost half the patients and high serum cryoglobulin levels, contrasting with a lower frequency of glomerulonephritis than expected (30%). The 1-, 3-, 5-, and 10-year survival rates were 97%, 94%, 94%, and 87%, respectively. Compared to MGUS, type I cryoglobulinaemia vasculitis related to haematological malignancy tended to be associated with a poorer prognosis. Therapeutic regimens based on alkylating agents, RTX, thalidomide, or lenalidomide, and bortezomib showed similar efficacy on vasculitis manifestations, with clinical response rates from 80% to 86%.

In an experimental model of murine monoclonal cryoglobulinaemia with MPGN, it was shown that cryoprecipitation is due to a particular γ3 constant region, although its nephrogenicity is abolished by a limited variation in the variable V_κ domain (Rengers et al., 2000). This suggests that cryoprecipitation and tissue deposition may be isotype dependent in animal models and human pathology.

Type I cryoglobulinaemic vasculitis is often life-threatening because of the severity of cutaneous and visceral involvement and the constant underlying malignant haematological disorder. Treatment of vasculitis is that of the haemopathy, but specific treatment may also be indicated, including plasma exchange, corticosteroids, RTX, or iloprost. The efficacy of RTX in type I cryoglobulinaemia is controversial. The absence of CD20 expression on plasma cells was supposed to explain a lack of efficacy. It has been also suggested that CD20 cross-linking could provoke cryoglobulin release through massive B-cell apoptosis or activation, which raises some issues as to the use of RTX in these patients.

Conclusion

Cryoglobulinaemia is a pathological condition characterized by the presence in the blood of a group of proteins showing the common property of precipitating from cooled serum. HCV is increasingly recognized as an instigator of B-cell lymphoproliferative disorders including MC. However, it remains unknown how exactly B cells activate, clonally expand, and differentiate to produce pathological quantities of self-reactive RF during the course of chronic HCV infection. Cryoglobulins are immune complexes that precipitate and deposit on vascular endothelium giving a small-sized vessel vasculitis in various organs, mainly the skin, kidneys, and peripheral nerves. A well-characterized pattern of glomerular disease termed 'cryoglobulinaemic glomerulonephritis' is frequent in individuals with HCV-associated MC; the most important histologic picture of cryoglobulinaemic glomerulonephritis is type I MPGN with subendothelial deposits. Recent advances have been made in the management of HCV-associated cryoglobulinaemic vasculitis with kidney involvement, and various approaches have been tried including optimal antiviral regimen, immunosuppressive therapy (corticosteroids, RTX, and cytotoxic agents), and plasma exchange. We recommend that the treatment of HCV-related MC vasculitis should be made according to the clinico-biological presentation.

References

Agnello, V., Chung, R., and Kaplan, L. (1992). A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med*, 327, 1490–5.

Alric, L., Plaisier, E., Thebault, S., *et al.* (2004). Influence of antiviral therapy in hepatitis C virus-associated cryoglobulinemic MPGN. *Am J Kidney Dis*, 43, 617–23.

Annear, N. M., Cook, H. T., Atkins, M., *et al.* (2010). Non-hepatitis virus associated mixed essential cryoglobulinemia. *Kidney Int*, 77, 161–4.

Arzoo, K., Sadeghi, S., and Liebman, H. A. (2002). Treatment of refractory antibody mediated autoimmune disorders with an anti-CD20 monoclonal antibody (rituximab). *Ann Rheum Dis*, 61, 922–4.

Asrani, S., Buchanan, P., Pinsky, B., *et al.* (2010). Lack of association between hepatitis C infection and chronic kidney disease. *Clin Gastroenterol Hepatol*, 8, 79–84.

Authier, F., Pawlotsky, J., Viard, J., *et al.* (1993). High incidence of hepatitis C virus infection in patients with cryoglobulinemic neuropathy. *Ann Neurol*, 34, 749–50.

Barsoum, R. (2007). Hepatitis C virus: from entry to renal injury—facts and potentials. *Nephrol Dial Transplant*, 22, 1840–8.

Basse, G., Ribes, D., Kamar, N., *et al.* (2006). Rituximab therapy for mixed cryoglobulinemia in seven renal transplant patients. *Transplant Proc*, 38, 2308–10.

Beddhu, S., Bastacky, S., and Johnson, J. P. (2002). The clinical and morphologic spectrum of renal cryoglobulinemia. *Medicine (Baltimore)*, 81, 398–409.

Braun A, Neumann T, Oelzner P, *et al.* (2008). Cryoglobulinaemia type III with severe neuropathy and immune complex

glomerulonephritis: remission after plasmapheresis and rituximab. *Rheumatol Int*, 28, 503–6.
Brouet, J., Clauvel, J., Danon, F., *et al.* (1974). Biologic and clinical significance of cryoglobulins. A report of 86 cases. *Am J Med*, 57, 775–88.
Bryce, A. H., Dispenzieri, A., Kyle, R. A., *et al.* (2006). Response to rituximab in patients with type II cryoglobulinemia. *Clin Lymphoma Myeloma*, 7, 140–4.
Bruchfeld, A., Lindahl, K., Stahle, L., *et al.* (2003). Interferon and ribavirin treatment in patients with hepatitis C-associated renal disease and renal insufficiency. *Nephrol Dial Transplant*, 18, 1573–80.
Butt, A., Wang, X., and Fried, L. (2011). HCV infection and the incidence of chronic kidney disease. *Am J Kidney Dis*, 57, 396–402.
Cacoub, P., Delluc, A., Saadoun, D., *et al.* (2008). Anti-CD20 monoclonal antibody (rituximab) treatment for cryoglobulinemic vasculitis: where do we stand? *Ann Rheum Dis*, 67, 283–7.
Cacoub, P., Fabiani, F. L., Musset, L., *et al.* (1994). Mixed cryoglobulinemia and hepatitis C virus. *Am J Med*, 96, 124–32.
Cacoub, P., Lidove, O., Maisonobe, T., *et al.* (2002). Interferon-alpha and ribavirin treatment in patients with hepatitis C virus-related systemic vasculitis. *Arthritis Rheum*, 46, 3317–26.
Cacoub, P., Maisonobe, T., Thibault, V., *et al.* (2001). Systemic vasculitis in patients with hepatitis C. *J Rheumatol*, 28, 109–18.
Cacoub, P., Poynard, T., Ghillani, P., *et al.* (1999). MULTIVIRC Group. Multidepartment virus C. Extra-hepatic manifestations of chronic hepatitis C. *Arthritis Rheum*, 42, 2204–12.
Cacoub, P., Renou, C., Rosenthal, E., *et al.* (2000). Extrahepatic manifestations associated with hepatitis C virus infection. A prospective multicenter study of 321 patients. The GERMIVIC. Groupe d'Etude et de Recherche en Medecine Interne et Maladies Infectieuses sur le Virus de l'Hepatite C. *Medicine (Baltimore)*, 79, 47–56.
Casato, M., Agnello, V., Pucillo, L. P., *et al.* (1997). Predictors of long-term response to high-dose interferon therapy in type II cryoglobulinemia associated with hepatitis C virus infection. *Blood*, 90, 3865–73.
Casato, M., Saadoun, D., Marchetti, A., *et al.* (2005). Central nervous system involvement in hepatitis C virus cryoglobulinemia vasculitis: a multicenter case-control study using magnetic resonance imaging and neuropsychological tests. *J Rheumatol*, 32, 484–8.
Cavallo, R., Roccatello, D., Menegatti, E., *et al.* (2009). Rituximab in cryoglobulinemic peripheral neuropathy. *J Neurol*, 256, 1076–82.
Charles, E., Green, R., Marukian, S., *et al.* (2008). Clonal expansion of immunoglobulin M^+CD27^+ B-cells in HCV-associated mixed cryoglobulinemia. *Blood*, 111, 1344–56.
Charles, E. and Dustin, L. (2009). Hepatitis C virus-induced cryoglobulinemia. *Kidney Int*, 76, 818–24.
Choo, Q., Kuo, G., Weiner, A., *et al.* (1989). Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*, 244, 359–62.
Cohen, H., Green, S., Jones, S., *et al.* (2007). Lack of efficacy of Rituximab in a patient with essential mixed cryoglobulinaemia. *Rheumatology (Oxford)*, 46, 366–7.
Costa, J., Resende, C., and de Carvalho, M. (2003). Motor-axonal polyneuropathy associated with hepatitis C virus. *Eur J Neurology*, 10, 183–5.
Cruzado, J., Carrera, M., Torras, J., *et al.* (2001). Hepatitis C virus infection and de novo glomerular lesions in renal allografts. *Am J Transplant*, 1, 171–8.
D'Amico, G. (1998). Renal involvement in hepatitis C infection: cryoglobulinemic glomerulonephritis. *Kidney Int*, 54, 650–71.
Dalrymple, L., Koepsell, T., Sampson, J., *et al.* (2007). Hepatitis C virus infection and the prevalence of renal insufficiency. *Clin J Am Soc Nephrol*, 2, 715–21.
Dammacco, F., Sansonno, D., Han, J. H., *et al.* (1994). Natural interferon-alpha versus its combination with 6-methyl-prednisolone in the therapy of type II mixed cryoglobulinemia: a long-term, randomized, controlled study. *Blood*, 84, 3336–43.
Dammacco, F., Tucci, F.A., Lauletta, G., *et al.* (2010). Pegylated interferon-alpha, ribavirin, and rituximab combined therapy of hepatitis C virus-related mixed cryoglobulinemia: a long-term study. *Blood*, 116, 343–53.
Della Rossa, A., Tavoni, A., D'Ascanio, A., *et al.* (2010). Mortality rate and outcome factors in mixed cryoglobulinaemia: the impact of hepatitis C virus. *Scand J Rheumatol*, 39, 167–70.
El-Serag, H., Hampel, H., Yeh, C., *et al.* (2002). Extra-hepatic manifestations of hepatitis C among United States male veterans. *Hepatology*, 36, 1439–45.
Fabrizi, F., Bruchfeld, A., Mangano, S., *et al.* (2007). Interferon therapy for HCV-associated glomerulonephritis: meta-analysis of controlled trials. *Int J Artif Organs*, 30, 212–19.
Fabrizi, F., Colucci, P., Ponticelli, C., *et al.* (2002). Kidney and liver involvement in cryoglobulinemia. *Semin Nephrol*, 22, 309–18.
Fabrizi, F., Pozzi, C., Farina, M., *et al.* (1998). Hepatitis C virus infection and acute or chronic glomerulonephritis: an epidemiological and clinical appraisal. *Nephrol Dial Transplant*, 13, 1991–7.
Ferri, C. (2008). Mixed cryoglobulinemia. *Orphanet J Rare Dis*, 3, 25.
Ferri, C., Sebastiani, M., Giuggioli, D., *et al.* (2004). Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. *Semin Arthritis Rheum*, 33, 355–74.
Ferri, C., Zignego, A. L., and Pileri, S. A. (2002). Cryoglobulins. *J Clin Pathol*, 55, 4–13.
Fornasieri, A., Li, M., Armelloni, S., *et al.* (1993). Glomerulonephritis induced by human IgM*k*-IgG cryoglobulins in mice. *Lab Invest*, 69, 531–40.
Gorevic, P., Kassab, H., Levo, Y., *et al.* (1980). Mixed cryoglobulinemia: clinical aspects and long-term follow-up of 40 patients. *Am J Med*, 69, 287–308.
Grcevska, L., Polenakovik, M., and Polenakovic, B. (1998). Renal involvement in essential monoclonal type 1 IgG cryoglobulinaemia. *Clin Nephrol*, 50, 200–2.
Hausfater, P., Cacoub, P., Assogba, U., *et al.* (2002). Plasma exchange and interferon-alpha pharmacokinetics in patients with hepatitis C virus-associated systemic vasculitis. *Nephron*, 91, 627–30.
Hezode, C., Forestier, N., Dusheiko, G., *et al.* (2009). Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med*, 360, 1839–50.
Huang, J., Chuang, W., Dai, C., *et al.* (2006). Viral hepatitis and proteinuria in an area endemic for hepatitis B and C infections: another chain of link? *J Intern Med*, 260, 255–62.
Invernizzi, F., Galli, M., Serino, G., *et al.* (1983). Secondary and essential cryoglobulinemias. Frequency, nosological classification, and long-term follow-up. *Acta Haematol*, 70, 73–82.
Johnson, R., Gretch, D., Couser, W., *et al.* (1994). Hepatitis C virus-associated glomerulonephritis. Effect of alpha-interferon therapy. *Kidney Int*, 46, 1700–4.
Karras, A., Noel, L., Droz, D., *et al.* (2002). Renal involvement in monoclonal (type I) cryoglobulinemia: two cases associated with IgG3k cryoglobulin. *Am J Kidney Dis*, 40, 1091–6.
Koukoulaki, M., Abeygunasekara, S. C., Smith, K. G., *et al.* (2005). Remission of refractory hepatitis C-negative cryoglobulinaemic vasculitis after rituximab and infliximab. *Nephrol Dial Transplant*, 20, 213–16.
Kuo, G., Choo, Q., Alter, H., *et al.* (1989). An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science*, 244, 362–4.
Landau, D. A., Rosenzwajg, M., Saadoun, D., *et al.* (2008). Correlation of clinical and virologic responses to antiviral treatment and regulatory T cell evolution in patients with hepatitis C virus-induced mixed cryoglobulinemia vasculitis. *Arthritis Rheum*, 58(9), 2897–907.
Landau, D. A., Saadoun, D., Calabrese, L. H., *et al.* (2007). The pathophysiology of HCV induced B-cell clonal disorders. *Autoimmun Rev*, 6, 581–7.
Landau, D. A., Scerra, S., Sene, D., *et al.* (2010). Causes and predictive factors of mortality in a cohort of patients with hepatitis C virus-related cryoglobulinemic vasculitis treated with antiviral therapy. *J Rheumatol*, 37, 615–21.
Lee, J., Lin, M., Yang, Y., *et al.* (2010). Association of hepatitis C and B virus infection with chronic kidney disease in an endemic area in Taiwan: a cross-sectional survey. *Am J Kidney Dis*, 56, 23–31.

Lerner, A. and Watson, C. (1947). Studies of cryoglobulins. I. Unusual purpura associated with the presence of a high concentration of cryoglobulin (cold precipitable serum globulins). *Am J Med*, 214, 410–18.

Liangpunsakul, S. and Chalasani, N. (2005). Relationship between hepatitis C and microalbuminuria: results from the NHANES III. *Kidney Int*, 67, 285–90.

Lunel, F., Musset, L., Cacoub, P., *et al.* (1994). Cryoglobulinemia in chronic liver diseases: role of hepatitis C virus and liver damage. *Gastroenterology*, 106, 1291–300.

Marukian, S., Jones, C., Andrus, L., *et al.* (2008). Cell culture-produced hepatitis C virus does not infect peripheral blood mononuclear cells. *Hepatology*, 48, 1843–50.

Matignon, M., Cacoub, P., Colombat, M., *et al.* (2009). Clinical and morphologic spectrum of renal involvement in patients with mixed cryoglobulinemia without evidence of hepatitis C virus infection. *Medicine (Baltimore)*, 88, 341–8.

McHutchison, J. G., Everson, G. T., Gordon, S. C., *et al.* (2009). Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med*, 360, 1827–38.

Meltzer, M., Franklin, E., Elias, K., *et al.* (1966). Cryoglobulinemia – a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. *Am J Med*, 40, 837–56.

Misiani, R., Bellavita, P., Fenili, D., *et al.* (1994). Interferon alfa-2a therapy in cryoglobulinemia associated with hepatitis C virus. *N Engl J Med*, 330, 751–6.

Montalbano, M., Pasulo, L., Sonzogni, A., *et al.* (2007). Treatment with pegylated interferon and ribavirin for hepatitis C virus-associated severe cryoglobulinemia in a liver/kidney transplant recipient. *J Clin Gastroenterol*, 41, 216–20.

Monti, G., Galli, M., Invernizzi, F., *et al.* (1995). Cryoglobulinaemias: a multi-centre study of the early clinical and laboratory manifestations of primary and secondary disease. GISC. Italian Group for the Study of Cryoglobulinaemias. *QJM*, 88, 115–26.

Naarendorp, M., Kallemuchikkal, U., Nuovo, G. J., *et al.* (2001). Long-term efficacy of interferon-alpha for extrahepatic disease associated with hepatitis C virus infection. *J Rheumatol*, 28, 2466–73.

Nehme-Schuster, H., Korganow, A. S., Pasquali, J. L., *et al.* (2005). Rituximab inefficiency during type I cryoglobulinaemia. *Rheumatology (Oxford)*, 44, 410–11.

Pandrangi, S., Singh. A., Wheeler, D. E., *et al.* (2008). Rituximab treatment for a patient with type I cryoglobulinemic glomerulonephritis. *Nat Clin Pract Nephrol*, 4, 393–7.

Ponticelli, C., Imbasciati, E., Tarantino, A., *et al.* (1977). Acute anuric glomerulonephritis in monoclonal cryoglobulinaemia. *Br Med J*, 1, 948.

Quartuccio, L., Fabris, M., Moretti, M., *et al.* (2008). Resistance to rituximab therapy and local BAFF overexpression in Sjogren's syndrome-related myoepithelial sialadenitis and low-grade parotid B-cell lymphoma. *Open Rheumatol J*, 2, 38–43.

Ramos-Casals, M., Robles, A., Brito-Zeron, P., *et al.* (2006). Life-threatening cryoglobulinemia: clinical and immunological characterization of 29 cases. *Semin Arthritis Rheum*, 36, 189–96.

Reininger, L., Berney, T., Shibata, T., *et al.* (1990). Cryoglobulinemia induced by a murine IgG3 rheumatoid factor: skin vasculitis and glomerulonephritis arise from distinct pathogenic mechanisms. *Proc Natl Acad Sci U S A*, 87, 1038–42.

Rengers, J., Touchard, G., Decourt, C., *et al.* (2000). Heavy and light chain primary structures control IgGC3 nephritogenicity in an experimental model for cryo-crystal-globulinemia. *Blood*, 95, 347–72.

Roccatello, D., Baldovino, S., Rossi, D., *et al.* (2004). Long-term effects of anti-CD20 monoclonal antibody treatment of cryoglobulinaemic glomerulonephritis. *Nephrol Dial Transplant*, 19, 3054–61.

Roccatello, D., Fornasieri, A., Giachino, O., *et al.* (2007). Multicenter study on hepatitis C virus-related cryoglobulinemic glomerulonephritis. *Am J Kidney Dis*, 49, 69–82.

Roccatello, D., Isidoro, C., Mazzucco, G., *et al.* (1993). Role of monocytes in cryoglobulinemia-associated nephritis. *Kidney Int*, 43, 1150–5.

Rosa, D., Saletti, G., De Gregorio, E., *et al.* (2005). Activation of naive B lymphocytes via CD81, a pathogenetic mechanism for hepatitis C virus-associated B lymphocyte disorders. *Proc Natl Acad Sci U S A*, 102, 18544–9.

Ruch, J., McMahon, B., Ramsey, G., *et al.* (2009). Catastrophic multiple organ ischemia due to an anti-Pr cold agglutinin developing in a patient with mixed cryoglobulinemia after treatment with rituximab. *Am J Hematol*, 84, 120–2.

Saadoun, D., Bieche. I., Maisonobe, T., *et al.* (2005). Involvement of chemokines and type 1 cytokines in the pathogenesis of hepatitis C virus-associated mixed cryoglobulinemia vasculitis neuropathy. *Arthritis Rheum*, 52, 2917–25.

Saadoun, D., Resche Rigon, M., Sene, D., *et al.* (2010). Rituximab plus Peg-interferon-alpha/ribavirin compared with Peg-interferon-alpha/ribavirin in hepatitis C-related mixed cryoglobulinemia. *Blood*, 116, 326–34.

Saadoun, D., Resche-Rigon, M., Thibault, V., *et al.* (2006a). Antiviral therapy for hepatitis C virus—associated mixed cryoglobulinemia vasculitis: a long-term follow-up study. *Arthritis Rheum*, 54, 3696–706.

Saadoun, D., Resche Rigon, M., Thibault, V., *et al.* (2014). Peg-IFNα/ribavirin/protease inhibitor combination in hepatitis C virus associated mixed cryoglobulinemia vasculitis: results at week 24. *Ann Rheum Dis*, 73(5), 831–7.

Saadoun, D., Rosenzwajg, M., Joly, F., *et al.* (2011a). Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. *N Engl J Med*. 365(22), 2067–77.

Saadoun D., Sellam, J., Ghillani-Dalbin, P., *et al.* (2006b). Increased risks of lymphoma and death among patients with non-hepatitis C virus-related mixed cryoglobulinemia. *Arch Intern Med*, 166, 2101–8.

Saadoun, D., Terrier, B., Semoun, O., *et al.* (2011b). Hepatitis C virus-associated polyarteritis nodosa. *Arthritis Care Res (Hoboken)*, 63(3), 427–35.

Sansonno, D., De Re, V., Lauletta, G., *et al.* (2003). Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon alpha with an anti-CD20. *Blood*, 101, 3818–26.

Sansonno, D., Gesualdo, L., Manno, C., *et al.* (1997). Hepatitis C virus-related proteins in kidney tissue from hepatitis C virus-infected patients with cryoglobulinaemic membranoproliferative glomerulonephritis. *Hepatology*, 25, 1237–44.

Sansonno, D., Lauletta, G., Montrone, M., *et al.* (2005). Hepatitis C virus RNA and core protein in kidney glomerular and tubular structures isolated with laser capture microdissection. *Clin Exp Immunol*, 140, 498–506.

Sene, D., Ghillani-Dalbin, P., Amoura, Z., *et al.* (2009). Rituximab may form a complex with IgMkappa mixed cryoglobulin and induce severe systemic reactions in patients with hepatitis C virus-induced vasculitis. *Arthritis Rheum*, 60, 3848–55.

Sene, D., Ghillani-Dalbin, P., Thibault, V., *et al.* (2004). Longterm course of mixed cryoglobulinemia in patients infected with hepatitis C virus. *J Rheumatol*, 31, 2199–206.

Sene, D., Limal, N., Ghillani-Dalbin, P., *et al.* (2007). Hepatitis C virus-associated B-cell proliferation—the role of serum B lymphocyte stimulator (BLyS/BAFF). *Rheumatology*, 46, 65–9.

Tarantino, A., Campise, R., Banfi, G., *et al.* (1995). Long-term predictors of survival in essential mixed cryoglobulinemic glomerulonephritis. *Kidney Int*, 47, 618–23.

Tarantino, A., De Vecchi, A., Montagnino, G., *et al.* (1981). Renal disease in essential mixed cryoglobulinemia. Long-term follow-up of 44 patients. *QJM*, 50, 1–30.

Terrier, B., Carrat, F., Krastinova, E., *et al.* (2013a). Prognostic factors of survival in patients with non-infectious mixed cryoglobulinaemia vasculitis: data from 242 cases included in the CryoVas survey. *Ann Rheum Dis*, 72(3), 374–80.

Terrier, B., Joly, F., Vazquez, T., *et al.* (2011a). Expansion of functionally anergic CD21-/low marginal zone-like B cell clones in hepatitis C virus infection-related autoimmunity. *J Immunol*, 187(12), 6550–63.

Terrier, B., Karras, A., Kahn, J. E., *et al.* (2013b). The spectrum of type I cryoglobulinemia vasculitis: new insights based on 64 cases. *Medicine (Baltimore)*, 92(2), 61–8.

Terrier, B., Krastinova, E., Marie, I., *et al.* (2012). Management of noninfectious mixed cryoglobulinemia vasculitis: data from 242 cases included in the CryoVas survey. *Blood*, 119(25), 5996–6004.

Terrier, B., Launay, D., Kaplanski, G., *et al.* (2010a). Safety and efficacy of rituximab in nonviral cryoglobulinemia vasculitis: data from the French Autoimmunity and Rituximab registry. *Arthritis Care Res (Hoboken)*, 62, 1787–95.

Terrier, B., Saadoun, D., Sene, D., *et al.* (2009). Efficacy and tolerability of rituximab with or without Pegylated interferon alfa-2b plus ribavirin in severe hepatitis C virus-related vasculitis: a long-term follow-up study of thirty-two patients. *Arthritis Rheum*, 60, 2531–40.

Terrier, B., Saadoun, D., Sène, D., *et al.* (2010b). Presentation and outcome of gastrointestinal involvement in hepatitis C virus-related systemic vasculitis: a case-control study from a single-centre cohort of 163 patients. *Gut*, 59, 1709–15.

Terrier, B., Semoun, O., Saadoun, D., *et al.* (2011b). Prognostic factors in patients with hepatitis C virus infection and systemic vasculitis. *Arthritis Rheum*, 63, 1748–57.

Trendelenburg, M., Fossati-Jimack, L., Cortes-Hernandez, J., *et al.* (2005). The role of complement in cryoglobulin-induced immune complex glomerulonephritis. *J Immunol*, 175, 6909–14.

Trejo, O., Ramos-Casals, M., Garcia-Carrasco, M., *et al.* (2001). Cryoglobulinemia, study of etiological factors and clinical and immunological features in 443 patients from a single center. *Medicine*, 80, 252–62.

Tsui, J., Vittinghoff, E., Shlipak, M., *et al.* (2006). Relationship between hepatitis C and chronic kidney disease: results from the Third National Health and Nutrition Examination Survey. *J Am Soc Nephrol*, 17, 1168–74.

Vallat, L., Benhamou, Y., Gutierrez, M., *et al.* (2004). Clonal B cell populations in the blood and liver of patients with chronic hepatitis C virus infection. *Arthritis Rheum*, 50, 3668–78.

Valli, G., De Vecchi, A., Gaddi, L., *et al.* (1989). Peripheral nervous system involvement in essential cryoglobulinemia and nephropathy. *Clin Exp Rheum*, 7, 479–83.

Viegi, G., Fornari, E., Ferri, C., *et al.* (1989). Lung function in essential mixed cryoglobulinemia: a short term-follow-up. *Clin Rheum*, 8, 331–8.

Wintrobe, M. and Buell, M. (1933). Hyperproteinemia associated with multiple myeloma: With report of a case in which an extraordinary hyperproteinemia was associated with thrombosis of the retinal veins and symptoms suggesting Raynaud's disease. *Bull Hopkins Hosp*, 52, 156–64.

Wornle, M., Schmid, H., Banas, B., *et al.* (2006). Novel role of toll-like receptor 3 in hepatitis C-associated glomerulonephritis. *Am J Pathol*, 168, 370–85.

Zaja, F., De Vita, S., Mazzaro, C., *et al.* (2003). Efficacy and safety of rituximab in type II mixed cryoglobulinemia. *Blood*, 101, 3827–34.

Zuckerman, E., Keren, D., Slobodin, G., *et al.* (2000). Treatment of refractory, symptomatic, hepatitis C virus related mixed cryoglobulinemia with ribavirin and interferon-alpha. *J Rheumatol*, 27, 2172–8.

CHAPTER 152

The patient with amyloidosis

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Introduction

Amyloidosis is a generic term for a group of diseases caused by misfolding and extracellular accumulation of certain proteins as fibrillar deposits that stain with Congo red and produce pathognomonic green birefringence when viewed by microscopy under crossed polarized light. The process of amyloid formation and deposition causes tissue toxicity and progressive organ dysfunction. Amyloidosis is remarkably diverse and can be hereditary or acquired, localized or systemic, and lethal or merely an incidental finding. So far almost 30 different human proteins with amyloidogenic potential have been identified of which 15 cause systemic amyloidosis, and the kidney is substantially involved in nine of the latter (Table 152.1). The different types of amyloid contain different fibril precursor proteins and are distinct diseases. Current management of amyloidosis is dependent on recognizing the type and the underlying condition which it complicates.

Without treatment, systemic disease is usually fatal but measures that reduce the supply of amyloid fibril precursor proteins can result in regression of amyloid deposits, prevention of organ failure, and improved survival.

Aetiology

Amyloidoses comprise a heterogeneous group of disorders in which a protein or peptide loses, or fails to acquire, its physiologic, functional folding and, in its misfolded state, undergoes fibrillization and extracellular deposition (Merlini and Bellotti, 2003). These deposits display distinctive ultrastructural (beta-sheet conformation) and tinctorial properties. The process of amyloid formation and deposition ultimately results in tissue damage and organ dysfunction (Fig. 152.1).

Basic research and clinical observations have identified some elements associated with the ability of a protein to form amyloid *in vivo* and give rise to disease, and these include the following:

- Pathologic and sustained increase in the concentration of a protein with increased propensity to aggregate. This is the case of the acute phase reactant serum amyloid A protein (SAA) in chronic inflammations and of β_2-microglobulin in patients with end-stage renal failure (ESRF).
- Mutations that destabilize a protein and favour its misfolding and aggregation, as occurs in hereditary amyloidoses
- Proteolytic remodelling of a protein, as in the case of the protease furin cleaving ABri and gelsolin and the β- and γ-secretases releasing amyloid-β (Aβ) peptides)
- Intrinsic propensity to misfolding, for instance, wild type transthyretin and apolipoprotein A-I are both associated with age-related amyloid deposition.

Frequently, a combination of these factors determines the amyloidogenicity of an individual protein. However, the inherent amyloidogenicity of a specific protein, per se, is not sufficient to account for amyloid deposition *in vivo*. Undetermined environmental and genetic factors must be involved in amyloidogenesis. For instance, only a minority of patients with long-lasting inflammation and persistent elevation of SAA levels develop amyloid A (AA) amyloidosis (Obici et al., 2009) and, similarly, the disease-associated Val30Met mutation of transthyretin shows significant variation in penetrance and clinical presentation among different ethnic groups and geographic areas (Saraiva, 2002).

Amyloid structure

Electron microscopy and X-ray diffraction analysis reveal that amyloid deposits are composed of rigid, non-branching fibrils with an average diameter of 7.5–10 nm and a cross-β super-secondary structure (Cohen and Calkins, 1959; Eanes and Glenner, 1968). More recently, refined structural studies of amyloid fibrils by solid-state nuclear magnetic resonance spectroscopy and microcrystals of small amyloid-like peptides by X-ray diffraction analysis have revealed a degree of structural variation (reviewed in Chiti and Dobson, 2006; Greenwald and Riek, 2010).

Common constituents of amyloid deposits

Serum amyloid P (SAP) component, a glycoprotein of the pentraxin family, binds all types of amyloid, independently of the protein of origin, through a specific binding motif, and protects amyloid fibrils from proteolytic degradation (Pepys et al., 1997). These properties make SAP a means of imaging amyloid deposits and an ideal therapeutic target (Bodin et al., 2010). Proteoglycans are also common in amyloid deposits and heparan sulphate (HS) proteoglycans, in particular, show similar kinetics of tissue deposition to that of fibrillar proteins. The role of HS in the genesis of amyloid deposits has been demonstrated in a transgenic experimental model in which the fragmentation of HS by heparanase resulted in resistance to the induction of AA amyloidosis (Li et al., 2005). HS accelerates the transition of the amyloid protein from the native state into the amyloidogenic partially folded state (Motamedi-Shad et al., 2009) and promotes the rate of fibril formation of amyloidogenic immunoglobulin light chains (LCs) (Ren et al., 2010; Martin and Ramirez-Alvarado, 2011) and of other proteins through selective binding to a basic motif, as shown for SAA (Elimova et al., 2009), transthyretin

Table 152.1 Most common forms of systemic amyloidosis with kidney involvement

Type	Fibril precursor	Note
AL	Light-chain V region fragments	Also known as primary, myeloma-associated
AA	(Apo) serum AA	Also known as secondary, reactive
ALect2	Leukocyte chemotactic factor 2	Sporadic, more common in Mexican Americans and South Asians
AApoAI	Apolipoprotein AI	Familial, tubular dysfunction
AApoAII	Apolipoprotein AII	Familial, tubular dysfunction
ALys	Lysozyme	Familial
AFib	Fibrinogen Aa-chain	Familial
AGel	Gelsolin	Familial
ATTR	Transthyretin	Familial, only certain variants, and kidney involvement is usually late

(Noborn et al., 2011), and amyloid-beta (Ancsin, 2003). Basic heparin-binding peptides have been reported to recognize murine and human amyloid deposits in both *in vivo* and *ex vivo* tissues and are potential radiotracers of amyloid deposits (Wall et al., 2011). Other common elements found in amyloid deposits are components of the extracellular matrix, such as laminin, elastin, entactin, and collagen IV.

Kinetics of fibril formation

In vitro studies have shown that amyloid fibril formation proceeds, in many instances, through a 'nucleated growth' mechanism, which is reminiscent of crystallization. Starting from a solution of monomeric proteins, there is an initial lag phase, once a critical nucleus has been generated, fibril formation begins and proceeds with very fast kinetics: any amyloidogenic precursor in its aggregation-prone conformation is rapidly incorporated into the growing fibrils (Chiti and Dobson, 2006). This seeding mechanism has clinical implications, since the process of amyloid clearance, following a response to therapy, usually leaves traces of 'seeds' in tissues. In the case of a disease relapse, these may trigger rapid re-accumulation of amyloid deposits (Hawkins and Pepys, 1990).

Organ tropism

In systemic amyloidosis the precursor protein circulates in the blood so potentially could access almost any organ or tissue. Nonetheless, specific amyloidogenic proteins tend to deposit predominantly in defined organs, for example, the kidney for fibrinogen Aα chain and leucocyte chemotactic factor 2, the peripheral nerves for the transthyretin Met30 variant, and the joints and bones for β_2-microglobulin. Several factors may contribute to determining the site of amyloid deposition: local protein concentration, interaction with collagen, tissue-specific glycosaminoglycans, pH, specific local proteolytic enzymes, or cellular receptors. For instance, it has been reported that the low-affinity interaction of β_2-microglobulin with type I collagen (prevalent in bones and joints) promotes amyloid generation (Relini et al., 2006), and that this is strongly enhanced in

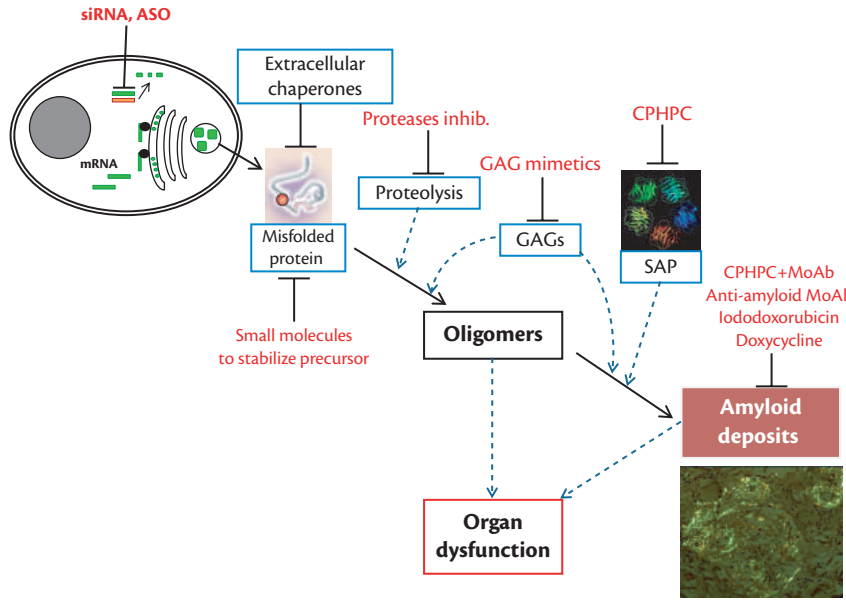

Fig. 152.1 Molecular events leading to amyloidosis. Interaction of the misfolded protein with the extracellular environment may result in proteolytic cleavage and binding to matrix components such as glycosaminoglycans (GAGs) and collagen that facilitate aggregation. Several lines of evidence support a role for extracellular chaperones in the *in vivo* clearance of aggregation-prone extracellular proteins. Serum amyloid P (SAP) binds to amyloid fibrils and protects them from reabsorption. The organ dysfunction may results from the combined action of the cytotoxic pre-fibrillar aggregates and of the amyloid deposits. Several new therapeutic approaches have been recently developed. The synthesis of the amyloid protein can be silenced using RNA interference (siRNA) or antisense oligonucleotides (ASO). Small molecules capable of stabilizing the amyloid precursor and preventing its misfolding and aggregation (diflunisal, tafamidis) are being tested in patients with ATTR amyloidosis. Inhibitors of proteases (secretase) are being evaluated in trials. Compounds interfering with the binding of GAGs to the amyloid proteins (eprodisate) have been tested successfully in patients with reactive amyloidosis. SAP can be cleared from amyloid deposits by using small palindromic drugs (CPHPC). The clearance of amyloid deposits can be promoted and accelerated by specific antibodies through passive and active immunotherapy. Small molecules, such as iododoxorubicin and doxycycline have shown to be able to disrupt the amyloid fibrils and have been tested in clinical trials.

the presence of heparin (Relini et al., 2008). In amyloid light-chain (AL) amyloidosis there is some evidence that the physicochemical characteristics (amino acid composition and conformation of the variable region) of the LC may be the most significant factor in determining the type and location of renal damage. LCs derived from the VL gene segment IGLV6-57 (previously named 6a) are preferentially associated with kidney involvement (Comenzo et al., 2001; Perfetti et al., 2002; Abraham et al., 2003) and LCs derived from germline IGLV1-44 are associated with heart targeting and damage (Perfetti et al., 2012). Amyloidogenic LCs are taken up by mesangial cells, transported to mature lysosomes for processing, and the resulting fibrils are then extruded into the extracellular matrix (Teng et al., 2004; Keeling and Herrera, 2005).

Mechanisms of tissue damage

These have not been fully elucidated; the presence of large amounts of amyloid material can disrupt tissue architecture and mechanically interfere with the physiologic function of affected organs (Pepys, 2006). However, compelling evidence also suggests that prefibrillar oligomeric species significantly contribute to organ dysfunction. Prefibrillar oligomers from transthyretin (Sousa et al., 2001; Andersson et al., 2002), Aβ (Lambert et al., 1998; Hartley et al., 1999; Walsh et al., 2002), immunoglobulin LCs (Liao et al., 2001; Brenner et al., 2004; Shi et al., 2010), and the prion protein (Silveira et al., 2005) have been shown to be toxic *in vitro* and/or *in vivo*. As a consequence of their conformational change, prefibrillar aggregates are predicted to expose hydrophobic portions that are normally buried inside the folded proteins or dispersed in the natively unfolded proteins. Recent *in vitro* studies confirm that structural flexibility and hydrophobic exposure are primary determinants of cytotoxicity (Campioni et al., 2010). Thus, organ damage may occur through intermingled mechanisms: the relative impact of amyloid deposits or prefibrillar aggregates on tissue dysfunction may vary among types of amyloidosis and organs.

Epidemiology

Systemic amyloidosis is a rare disease accounting for approximately 1 in 1500 deaths in the United Kingdom and presumably other developed countries. Although cases of amyloidosis have been reported in children it is predominantly a disease of mid to late life and accounts for 4% of adult renal biopsies (Mesquita et al., 2011) and 1.6% of patients starting dialysis (Tufveson et al., 1989).

Systemic amyloidosis associated with monoclonal light chains, AL amyloidosis

The age-adjusted incidence of AL amyloidosis in the United States has been estimated to be between 5.1 and 12.8 per million people per year and AL is the diagnosis in up to 85% renal biopsies containing amyloid (Said et al., 2013). Approximately 60% of cases are men and median age at presentation is 65 years, it can occur in young adults and is probably under-diagnosed in the elderly, in whom monoclonal gammopathies are most prevalent. AL amyloidosis develops in about 2% of individuals with monoclonal B-cell dyscrasias (Kyle et al., 2002). The B-cell dyscrasias underlying systemic AL amyloidosis can include almost any clonal proliferation of differentiated B lymphocytes, 94% have an underlying clone of plasma cells (Kyle and Gertz, 1995; Wechalekar et al., 2008). The clonal cell burden in AL amyloidosis is usually small (Obici et al., 2005; Merlini and Stone 2006) and the plasma cell proliferation fraction similar to monoclonal gammopathy of unknown significance (Witzig et al., 1999). Only 10–20% of patients who are diagnosed with AL amyloidosis meet myeloma criteria (Perfetti et al., 1999). Progression of the underlying monoclonal gammopathy to overt myeloma is rare in systemic AL amyloidosis (Rajkumar et al., 1998), which, in part reflects patients' short survival.

Reactive systemic, AA, amyloidosis

The exact incidence of AA amyloidosis is unclear but it accounts for 7–15% of the cases of amyloidosis seen at major referral centres. It is always a complication of inflammation and the list of chronic disorders that can be complicated by AA amyloidosis is summarized in Box 152.1. In industrialized countries inflammatory arthritides underlie 60% of cases. The prevalence of

Box 152.1 Inflammatory conditions which have been reported to underlie AA amyloidosis

Chronic inflammatory arthritides

- Rheumatoid arthritis
- Juvenile inflammatory arthritis
- Ankylosing spondylitis
- Psoriatic arthropathy
- Reiter's syndrome
- Adult Still disease.

Vasculitides

- Polyarteritis nodosa
- Takayasu arteritis
- Behçet disease
- Systemic lupus erythematosus
- Giant cell arteritis/polymyalgia rheumatic.

Chronic infections

- Bronchiectasis
- Chronic cutaneous ulcers
- Chronic pyelonephritis
- Chronic osteomyelitis
- Subacute bacterial endocarditis
- Leprosy
- Tuberculosis
- Whipple disease.

Inflammatory bowel disease

- Crohn disease
- Ulcerative colitis.

Periodic fevers

- Familial Mediterranean fever
- Cryopyrin-associated periodic syndrome (CAPS)
- TNF receptor-associated periodic syndrome (TRAPS)
- Mevalonate kinase deficiency (MVK).

Neoplasia

- Hodgkin disease
- Renal cell carcinoma
- Adenocarcinoma of the lung, gut, urogenital tract
- Basal cell carcinoma
- Hairy cell leukaemia
- Castleman disease
- Hepatic adenoma.

Other

- IV and subcutaneous drug abuse
- Cystic fibrosis
- Kartagener syndrome
- Epidermolysis bullosa
- Hypogammaglobulinaemia
- Cyclic neutropenia
- Common variable immunodeficiency
- Hyperimmunoglobulin M syndrome
- SAPHO syndrome.

AA amyloidosis in patients with chronic arthritides is between 3.6% and 5.8% (Schnitzer and Ansell, 1977; de Beer et al., 1982; Myllykangas-Luosujärvi et al., 1999). For unexplained reasons the incidence of AA amyloid is much lower in the United States than in Europe (Filipowicz-Sosnowska et al., 1978; Svantesson et al., 1983) and the incidence appears to be falling in Europe (Laiho et al., 1999). The median latency between onset of inflammation and diagnosis of amyloid is approximately 17 years but this varies from less than a year to decades. The median age at diagnosis is 50 years, presentation in childhood, although becoming less common, is still recognized. As with all types of amyloidosis, AA appears slightly commoner in men who account for 56% of the largest characterized series (Lachmann et al., 2007).

Dialysis-related amyloidosis

β_2-microglobulin amyloidosis occurs in patients who have been on dialysis for > 6–10 years, or very occasionally in individuals with longstanding, severe chronic renal impairment. Relatively few patients have been maintained on peritoneal dialysis for the 5–10 years required to develop symptomatic β_2-microglobulin amyloid, but histological studies of early subclinical deposits suggests that the incidence of dialysis-related amyloidosis (DRA) is similar among patients receiving the two dialysis modalities (Ballardie et al., 1986). β_2-microglobulin amyloid deposits have been reported in 20–30% of patients within 3 years of commencing dialysis for ESRF (Jadoul, 1998) but the incidence seems to fallen by > 80% due to advances in dialysis technology (Schwalbe et al., 1997; Schiffl, 2014).

Hereditary systemic amyloidosis

In the United Kingdom, the prevalence of hereditary non-neuropathic amyloidosis appears to be in the order of 1.5 per million with most patients presenting in their sixth decade. Hereditary systemic amyloidosis accounted for 1.9% of a recent large North American series of renal biopsies containing amyloid (Said et al., 2013).

LECT2 amyloidosis

Leukocyte chemotactic factor 2 (LECT2) amyloidosis is thought to account for up to 2.7% of renal biopsies containing amyloid (Murphy et al., 2010; Said et al., 2013).

Clinical features

Systemic amyloidosis associated with monoclonal light chains, AL amyloidosis

The clinical features of AL amyloidosis are protean (Merlini et al., 2011) as any organ other than the central nervous system can be directly involved:

- Many patients present with non-specific symptoms such as fatigue and weight loss.
- Cardiac involvement is a major determinant of outcome and occurs in 74% of patients at presentation, with approximately 30% presenting with congestive heart failure. Cardiac biomarkers provide a quantitative assessment of cardiac damage (troponin I or T) and wall strain (brain natriuretic peptide (BNP), N-terminal (NT)-proBNP) and are the most important predictors of outcome in amyloidosis (Dispenzieri et al., 2003; Palladini et al., 2003). By using the cut-offs of 0.035 micrograms/L for troponin T and 332 ng/L for NT-proBNP, patients can be classified into three stages (Dispenzieri et al., 2004), which are useful in selecting therapies and patient stratification in clinical trials.
- Renal dysfunction is seen in > 60% of cases and causes proteinuric renal failure in the context of a normal or low blood pressure. In recent large studies, 44% of patients presented with chronic kidney disease (CKD) stage 1 or 2, and 16% with CKD stage 5; median proteinuria was 5–7 g/day and median serum albumin 21–28 g/L (Gertz et al., 2009; Pinney et al., 2011).
- Hepatic amyloid is found in 54% of patients. Despite often substantial hepatomegaly, liver function is generally well preserved with modest elevation of alkaline phosphatase (median of 154 IU/L). Hyperbilirubinaemia is unusual but associated with a poor outcomes and a median survival of 4 months (Lovat et al., 1998).
- Gut involvement may cause motility disturbances (often secondary to autonomic neuropathy), malabsorption, perforation, haemorrhage, or obstruction.

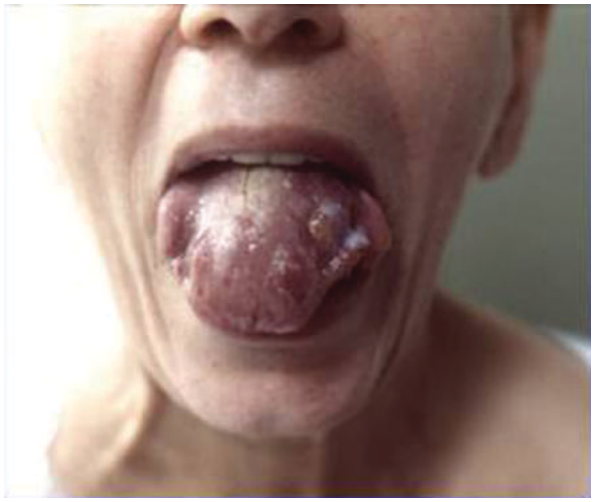

Fig. 152.2 Macroglossia is seen in approximately 10% of cases of AL amyloidosis.

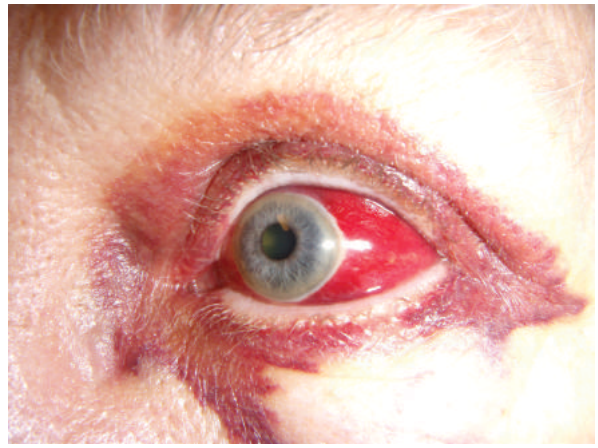

Fig. 152.3 Capillary fragility manifesting as periorbital bruising and a conjunctival haemorrhage in AL amyloidosis.

- Painful sensory polyneuropathy with early loss of temperature sensation followed later by motor deficits is seen in 10–20% of cases and carpal tunnel syndrome in 20%.
- Autonomic neuropathy leads to orthostatic hypotension, impotence, and gastrointestinal motility disturbances.
- Macroglossia occurs in 10% and is pathognomonic of AL type (Fig. 152.2).
- Skin involvement is common and usually takes the form of bruising spontaneously or after minor trauma (Fig. 152.3).
- Hyposplenism sometimes causes blood film abnormalities.
- An acquired bleeding diathesis may be associated with deficiency of factor X and factor IX, or with increased fibrinolysis.
- Articular amyloid is rare and may superficially resemble acute polyarticular arthritis, or it may present as asymmetrical arthritis affecting the hip or shoulder. Infiltration of the glenohumeral joint and surrounding soft tissues occasionally produces the characteristic 'shoulder pad' sign.

Reactive systemic, AA, amyloidosis

The predominant clinical manifestations of AA amyloidosis are renal:

- More than 97% of patients present with proteinuric kidney dysfunction. Haematuria, tubular defects, and diffuse renal calcification occur rarely. Just over 50% of patients have nephrotic syndrome at presentation. Approximately 10% of patients are in ESRF at diagnosis and > 40% eventually progress to ESRF (Lachmann et al., 2007).
- The spleen is almost always infiltrated.
- Adrenal glands are involved in > 33% although clinical hypoadrenalism is rare.
- Hepatosplenomegaly is seen at presentation in 9% of cases but liver failure is exceptionally rare.
- Malabsorption occurs only in very advanced disease.
- Cardiac amyloidosis is seen in 2% and only in advanced disease.

Dialysis-related amyloidosis

β_2-microglobulin amyloidosis is preferentially deposited in articular and peri-articular structures, and its manifestations are largely confined to the locomotor system (Drueke and Massy 2009):

- Carpal tunnel syndrome is usually the first clinical manifestation. Some individuals develop symptoms within 3–5 years of initiation of renal replacement therapy and by 20 years the prevalence was almost 100% (Bardin et al., 1986). Older patients appear to be more susceptible to the disease, and tend to exhibit symptoms more rapidly (Jadoul, 1998).
- Amyloid arthropathy tends to occur a little later but eventually affects the most patients on dialysis. It affects the shoulders, knees, wrists, and small joints of the hand and is associated with joint swelling, chronic tenosynovitis, and, occasionally, haemarthroses. Spondyloarthropathies are also well recognized, as is cervical cord compression. Deposition within the periarticular bone produces typical appearances of subchondral erosions and cysts which can contribute to pathological fractures particularly of the femoral neck, cervical vertebrae, and scaphoid.

LECT2 amyloidosis

Most patients present in the sixth to seventh decades with slowly progressive renal impairment. Proteinuria tends to be low grade and hypertension is well recognized. Although splenic and adrenal amyloid deposits are visible on SAP imaging, clinically the disease appears to be renal isolated.

Hereditary non-neuropathic systemic amyloidosis

Lysosyme

Most patients present in middle age with proteinuria, very slowly progressive renal impairment, and sometimes hepatosplenomegaly with or without purpuric rashes. In retrospect most recollect a long history of dry eyes and dry mouth. Substantial gastrointestinal amyloid deposits are common and important since gastrointestinal haemorrhage or perforation is a frequent cause of death in these patients.

Apolipoprotein A1

Depending on the mutation, patients can present with massive visceral amyloid involvement, predominant cardiomyopathy, or neuropathy. Most patients eventually develop renal failure and despite extensive amyloid deposition, liver function usually remains preserved. Amyloidosis caused by the ApoA1 Leu75Pro mutation has a very unusual phenotype characterized by moderate polyuria and negative urinalysis with evidence of defective urine-concentrating capacity and mild tubular proteinuria. The amyloid deposits are localized to the renal medulla unlike most other systemic amyloidoses which tend to be characterized by glomerular and vascular deposits. Additional features are hypertension, cholestatic hepatopathy, and primary hypogonadism with infertility. This atypical phenotype means that the diagnosis can easily be overlooked (Obici et al., 2004; Gregorini et al., 2005).

Fibrinogen A alpha chain

Patients with this form of hereditary amyloidosis frequently do not give a family history of similar disease and are readily misdiagnosed as having AL amyloid. Most patients present in their sixth to seventh decades with proteinuria or hypertension and progress to ESRF over 4–10 years. Amyloid deposition is seen in the kidneys, characteristically localized to the glomeruli, spleen, and sometimes the liver but is usually asymptomatic in the latter two sites.

Apolipoprotein A2

The few kindreds described have slowly progressive proteinuric renal failure.

Gelsolin

This usually presents with corneal lattice dystrophy and progressive cranial neuropathy. Renal amyloid deposits are often subclinical but can cause ESRF.

Transthyretin Met30

In addition to neuropathy and cardiac involvement up to a third of cases have evidence of proteinuria and renal failure and 10% eventually develop ESRF (Lobato et al., 2003). In addition, autonomic neuropathy causes impaired bladder emptying requiring indwelling urinary catheters with a risk of infection.

Investigations

Detection and typing of amyloid deposits

Diagnosis relies on a high index of clinical suspicion; unfortunately amyloid is frequently asymptomatic until a relatively late stage and can then present with highly variable or non-specific symptoms. Early diagnosis is essential in order to start therapy when organ function is still preserved or recoverable. Amyloidosis should be suspected in any patient with non-diabetic albuminuria; increased NTproBNP in the absence of primary heart disease; non-ischaemic cardiomyopathy with an echocardiogram suggesting concentric hypertrophy and presence of low electrocardiogram (ECG) voltages; hepatomegaly or increased alkaline phosphatase without an imaging abnormality; peripheral and/or autonomic neuropathy; unexplained facial or neck purpura or macroglossia. Any patient with suggestive features should undergo a biopsy looking for amyloid deposits.

Histology

The diagnosis of amyloidosis requires histological confirmation (Rocken et al., 1996) (Fig. 152.4). Subcutaneous fat aspiration will identify amyloid deposits in approximately 90% of patients (van Gameren et al., 2006) and biopsy of the labial salivary glands may detect amyloid deposits in 50% of patients with negative fat analysis. There have been concerns that organ biopsies carry an increased risk of haemorrhage. Although significant bleeds have been reported in 5% of liver biopsies, renal biopsies appear as safe in amyloid as other diagnoses (Fish et al., 2010). Congo red staining of amyloid produces pathognomic apple green birefringence when viewed under cross-polarized light and negatively stained electron microscopy reveals 10–15 nm diameter, rigid, non-branching fibrils composed of twisted protofibrils of indeterminate length.

The main protein constituting the amyloid deposit can be identified with immunohistochemistry, although this may be unreliable in AL and hereditary amyloidosis, or with immunogold techniques (Arbustini et al., 1997). Mass spectrometry can confirm the amyloid protein composition and will likely become the gold standard for identifying amyloid deposits (Lavatelli et al., 2008; Vrana et al., 2009; Sethi et al., 2013).

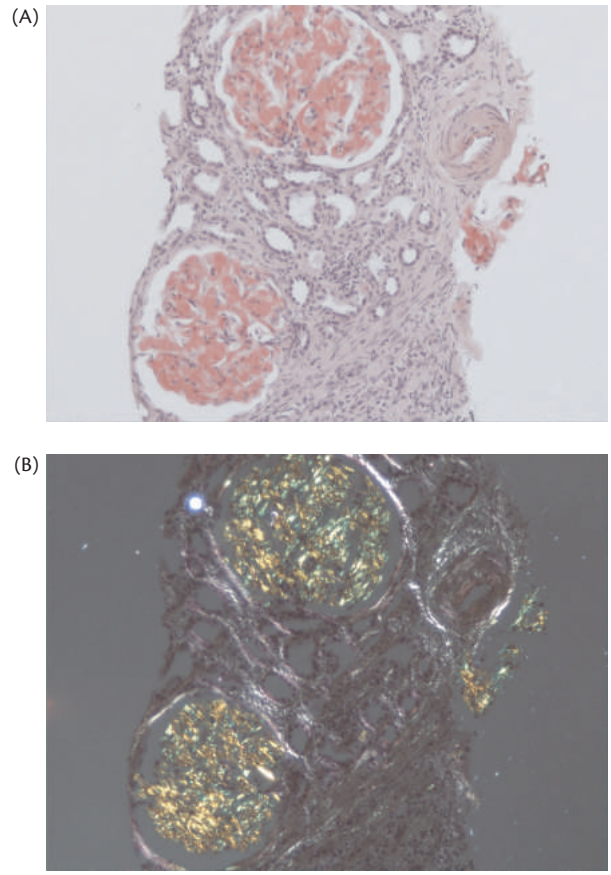

Fig. 152.4 Sections of renal biopsy stained with Congo red viewed under ×10 magnification. (A) Amorphous deposits of eosinophil material are seen within the glomeruli. (B) Pathognomonic apple green birefringence of the amyloid deposits when the section is view under cross-polarized light.

Imaging amyloid deposits

SAP scintigraphy

SAP concentrates specifically in amyloid deposits of all types as a result of its calcium-dependent binding to the fibrils. Radiolabelled

SAP scintigraphy has been used since 1988 in the United Kingdom for diagnosis and quantitative monitoring of amyloid deposits (Hawkins et al., 1988). This safe non-invasive method provides information on the presence, distribution, and extent of visceral amyloid deposits, and serial scans monitor progress and response to therapy. Unfortunately the method is not informative about amyloid deposition in the moving heart and is not commercially available.

Imaging the heart

Two-dimensional Doppler echocardiography classically reveals concentric biventricular wall thickening with a restrictive filling pattern and left ventricular longitudinal strain abnormalities. Amyloid causes diastolic dysfunction with well-preserved contractility until a very late stage (Falk, 2005). The ECG in advanced disease commonly shows small voltages and pathological 'Q' waves (pseudo-infarct pattern). Recent developments in magnetic resonance imaging have made a major contribution to the diagnosis of cardiac amyloidosis (Maceira et al., 2005). Scans using the bone tracer DPD are useful in detecting heart involvement in transthyretin amyloidosis (ATTR) (Rapezzi et al., 2011).

DNA analysis

Hereditary amyloidoses are rare and often overlooked. Although all types are dominantly inherited, penetrance and expressivity are highly variable and there is frequently no obvious family history. DNA analysis is mandatory in all patients in whom hereditary amyloidosis is suspected on the basis of clinical and laboratory features and in the rare cases in whom the type cannot be confirmed by immunohistochemistry or mass spectrometry. Multiple amyloidogenic mutations have been identified in the genes associated with hereditary amyloidosis and new variants are regularly identified (Benson, 2005).

Investigation of the underlying disease

AL amyloidosis

In all patients with diagnosed or suspected AL amyloidosis a search must be made for an underlying plasma cell clone. This should include serum and urine electrophoresis and immunofixation and serum free light chain (FLC) assay (Merlini et al., 2011, 2013). If a monoclonal protein is present, a bone marrow examination and bone imaging should be performed to exclude the presence of multiple myeloma. A bone marrow biopsy should also be stained with Congo red as the stroma or blood vessels will contain amyloid in > 50% of patients.

AA amyloidosis

In AA amyloidosis, it is necessary to identify the underlying inflammatory disease and specific investigation needs to be driven by the individual clinical features. The identification of the underlying disease may be very difficult due to the diverse conditions involved (see Box 152.1), and in approximately 10% of patients seen at UK and Italian centres the cause remains undetermined.

Treatment and outcome

Principles of treatment

Amyloid will regress if its deposition is slowed or its clearance is enhanced. Novel therapies with the latter aim are under development, but at present the treatment of all types of amyloid centres on reducing the supply of the amyloid precursor protein and supporting or replacing compromised organ function. As a result, treatment depends on precise identification of the amyloid fibril type. Early diagnosis is the key to effective therapy.

Systemic AL amyloidosis

The goals of therapy are prompt elimination of the misfolded amyloidogenic LCs, minimization of treatment toxicity, and support of the function of target organs. Updated consensus criteria for haematologic and organ responses in amyloidosis have been recently published (Palladini et al., 2012). Achieving a haematologic response translates into improved overall survival. Although partial responses can be beneficial (Lachmann et al., 2003), complete clonal responses are associated with the best clinical outcomes (Gertz et al., 2007). A new paradigm for the treatment of AL amyloidosis has been proposed in which both the underlying haematologic disorder and the end-organ damage can be monitored with FLC and cardiac biomarkers to optimize therapy and minimize toxicity (Merlini et al., 2013).

Treatment for AL amyloidosis is highly individualized, based on age, cardiac staging, and regimen toxicities, and should be response tailored with prompt regimen switch in case of no response to two to three cycles of treatment (recently reviewed in Merlini et al., 2011, 2013).

The outcomes of AL amyloidosis have improved following introduction of effective chemotherapy regimens during the last decade (Merlini et al., 2013), with response rates approaching 80% and median survival exceeding 5 years in the last cohorts (Merlini, 2012).

Response to therapy in patients with renal involvement

Median survival in patients presenting with renal disease is 26.8–35.2 months in two studies including a total of 1068 patients (Gertz et al., 2009; Pinney et al., 2011). Survival is strongly influenced by the degree of haematological response and the presence of cardiac amyloidosis but not by the degree of renal dysfunction at presentation. More than 40% of patients eventually received dialysis and 13–26% of cases presenting with potentially salvageable renal function (variously defined by baseline clearance of > 20 mL/min or baseline creatinine of < 5 mg/dL) progress to ESRF within a median of 12 months. Renal function deteriorated in almost 55% within a median of 24 months in one study; conversely renal function improves in approximately one-third of cases. CKD stage at baseline does not significantly influence renal response whereas a > 90% FLC response to chemotherapy is associated with an almost fourfold increase in renal response ($P < 0.001$) and a 68% reduction in the risk of renal progression ($P < 0.001$). In a study of 141 patients who had received stem cell transplantation, superior overall survival was seen in the 58% of patients who achieved a > 75% reduction in proteinuria. In these patients a rise in serum creatinine > 25% was not associated with a poorer outcome. Better haematologic responses were predictive of higher rates of proteinuria reduction (Leung et al., 2013). High-dose melphalan and stem cell rescue has been associated with renal toxicity with a doubling of serum creatinine seen in 23% but persistent renal decline in only a fifth of these patients (Dember et al., 2001). The potential nephrotoxicity

of lenalidomide has been recently reported and demands careful follow-up of renal function (Specter et al., 2011).

Reactive systemic, AA, amyloidosis

In AA amyloidosis, the aim of treatment is the complete prolonged control of the underlying inflammatory disease. The choice of therapy depends on the underlying disease process but therapeutic success must always be assessed by the long-term control of the acute phase response ideally by serial SAA measures or otherwise by C-reactive protein. Most patients with inflammatory arthritis have previously failed to respond to conventional disease modifying antirheumatoid drugs and many do well with anti-tumour necrosis factor (TNF) therapies or other biologics such as anti-CD20 antibodies or anti-interleukin (IL)-1 or IL-6 therapies. In patients who fail to respond to these agents there may still be a role for therapy with alkylating agents such as chlorambucil or cyclophosphamide (Berglund et al., 1993).

Median SAA levels have been shown to be strongly associated with both survival and renal outcome; persistent complete suppression of inflammation with normal SAA levels is associated with an almost 18-fold lower risk of death than median SAA levels of > 155 mg/L (Lachmann et al., 2007). Median survival of 79–137 months has been recently reported in large series from Italy (Bergesio et al., 2008) and the United Kingdom. Approximately 40% of patients will eventually require renal replacement therapy with a median time to dialysis of 78 months.

Dialysis-related amyloidosis

The only effective treatment for DRA is successful renal transplantation, although drugs targeting the amyloid deposits, such as doxycycline, are being tested (Montagna et al., 2013). Serum levels of β_2-microglobulin fall rapidly following transplantation and this is usually accompanied by an improvement in symptoms. This rapid response is probably due more to the anti-inflammatory properties of transplant immunosuppression and to discontinuation of dialysis. In contrast to the symptoms, radiological bone cysts heal slowly (Tan et al., 1996), and amyloid can be demonstrated histologically many years after renal transplantation. Attempts have been made to reduce DRA by altering the dialysis prescription. There is some evidence that the risks of DRA are increased in patients dialysed using less 'biocompatible' membranes, and that use of the more permeable membrane systems is relatively protective (Miyata et al., 1998). Greater removal of β_2-microglobulin is attained in patients undergoing high-flux haemodiafiltration and using column absorbing of this protein, and translates in improved articular symptoms and in delayed DRA (Traut et al., 2007; Gejyo et al., 2013; Schiffl, 2014). In addition, the incidence of DRA seems to be falling, perhaps linked to fewer contaminants in the dialysate (Schwalbe et al., 1997; Yamamoto and Gejyo, 2005). Surgery may be required to relieve carpal tunnel compression, stabilize the cervical spine, or to treat bone fractures.

Hereditary non-neuropathic systemic amyloidosis

These diseases, particularly lysozyme and ApoAI amyloidosis, tend to run very indolent courses and when renal failure is reached transplantation can be successful with grafts surviving for decades. The rate of renal deterioration seems to be faster in fibrinogen amyloidosis and the limited experience of renal transplantation suggests that amyloid deposition will cause graft loss in a median of 7 years (Gillmore et al., 2009). As fibrinogen is synthesized solely in the liver, combined hepato-renal transplantation offers the possibility of 'surgical gene therapy' and complete protection from recurrent amyloidosis. The limitation of this approach is the serious risks associated with combined transplantation (Stangou et al., 2010).

Preservation and replacement of organ function

Organs infiltrated by amyloid may fail acutely, often without obvious provocation. Attention must to be paid to salt and water balance, maintenance of the circulating volume, and prompt treatment of sepsis to reduce the risk of acute organ failure. Potentially nephrotoxic drugs, elective surgery, and general anaesthesia are best avoided unless there are compelling indications.

Significant renal disease is present at diagnosis in at least 75% of patients with systemic amyloidosis (Dember, 2006). Nephrotic syndrome generally requires treatment with high doses of loop diuretics and resistant cases may require addition of thiazide and/or potassium-sparing diuretics. Salt and, in many cases, fluid restriction may be advisable. In patients who have difficulty maintaining their intravascular volume, infusions of salt-poor human albumin can be very helpful.

Caution is required in the use of standard heart failure medications in patients with amyloidosis. Digoxin and calcium channel blockers have been associated with excess toxicity. Angiotensin-converting enzyme inhibitors can promote hypotension and should generally be avoided. Prophylactic amiodarone has been incorporated into therapy trials of amyloidosis to reduce the risk of sudden cardiac death if complex ventricular arrhythmias are detected on Holter ECG (Palladini et al., 2004). The use of beta blockers in patients with cardiac amyloid is associated with a higher mortality rate (Soni and LeLorier, 2005). Diuretics are the mainstay of therapy, but should be used with caution as amyloidosis causes a restrictive cardiomyopathy and high filling pressures are required to maintain cardiac output. Alpha agonists such as midodrine can improve orthostatic hypotension. Implantable cardiac defibrillators as well as left ventricular assist devices have been used, but their efficacy in this disease remains controversial (Kristen et al., 2008; Swiecicki et al., 2013).

In highly selected younger patients with isolated irreversible cardiac failure, heart transplantation offers a possibility of long-term survival and has been performed in a small number of patients (Dubrey et al., 2001; Gillmore et al., 2006; Maurer et al., 2007; Dey et al., 2010). The scarcity of donor hearts, the high transplant-related mortality, and the risk of amyloid deposition in the graft make rigorous patient selection mandatory. In AL amyloidosis, chemotherapy is required after cardiac transplantation to prevent graft amyloid or its progression in other organ systems.

Renal dialysis

The outcome of AL amyloidosis patients on long-term dialysis is improving but survival remains about 70% of that age-matched non-diabetic patients with other diseases (Gertz et al., 2009; Lachmann et al., 2010). Patients who commenced dialysis after 2002 in the United Kingdom had a median survival of 43.6 months whereas data from the United States and Italy report median survival of 10.4–11 months. The outcome in patients with other types of amyloid is more favourable (Bergesio et al., 2008; Immonen et al., 2008). In AA amyloidosis, median survival on dialysis has been reported between 17 months in earlier

series and 69 months; the latter in a cohort of 129 patients with an incident mortality of 18% and < 10% mortality in subsequent years (Lachmann et al., 2010). Recent data on 490 patients with amyloidosis of undifferentiated types from Australia and New Zealand are less favourable with a median survival of 2.09 years, 47% of that seen in other causes of renal failure with no significant survival difference between haemodialysis and peritoneal dialysis (Tang et al., 2013).

Renal transplantation

Although early mortality is increased, due to sepsis and cardiac failure, long-term renal graft survival and rejection rates comparable with other systemic diseases have been reported (Sattianayagam et al., 2009). Less than 10% of patients who reached ESRF due to AL amyloidosis receive a renal transplant; median patient and graft survival in these highly selected patients was 89 months (Pinney et al., 2011). In a few cases renal transplantation has been followed by autologous stem cell transplantation with stable renal function in 4/5 patients (Leung et al., 2005). Recent experience of renal transplantation in selected patients with AA amyloidosis has shown 5- and 10-year graft survival of 74% and 68% respectively. These encouraging results have led to some patients receiving living donor renal transplants (Sherif et al., 2003; Emiroglu et al., 2005). Most patients have a functioning graft until death (Jacob et al., 1979; Pinney et al., 2011), even though, in all types, amyloid deposition in the transplant is recognized (Gillmore et al., 2000, 2006).

Novel therapies and perspectives

The remarkable advances in the understanding of the molecular mechanisms involved in amyloid formation and tissue damage achieved in the last decade, have revealed several new drug targets (Fig. 152.1). Several new approaches have been developed:

- Synthesis of the amyloidogenic precursor may be silenced by RNA interference (Phipps et al., 2010) or by antisense oligonucleotides (Benson et al., 2006; Kluve-Beckerman et al., 2011).
- Inhibitors of proteases (secretase) are being evaluated in trials (Schenk et al., 2001).
- Inhibitors of glycosaminoglycans binding to the amyloid proteins (eprodisate) have been successful in secondary amyloidosis (Dember et al., 2007).
- Small molecules capable of stabilizing the amyloid precursor and preventing its misfolding and aggregation (diflunisal, tafamidis) (Obici et al., 2012) have been tested in ATTR amyloidosis leading to licensing of a novel agent, tafamidis (Coelho et al., 2012).
- SAP can be cleared from amyloid deposits by using small palindromic drugs (e.g. CPHPC) (Pepys et al., 2002).
- The clearance of amyloid deposits can be promoted and accelerated by specific antibodies through passive (Solomon et al., 2003. Wall et al., 2012) and active immunotherapy (Klyubin et al., 2005), or by combining CPHPC with anti-SAP antibodies (Bodin et al., 2010).
- Small molecules, such as iododoxorubicin (Merlini et al., 1995) and doxycycline (Almeida and Saraiva, 2012) are able to disrupt the amyloid fibrils and reduce the amyloid burden.

Clinicians should be aware that in the near future amyloid diseases will be treated with combination approaches that reduce protein precursor production, prevent aggregation, and induce fibril resorption.

References

Abraham, R. S., Geyer, S. M., Price-Troska, T. L., *et al.* (2003). Immunoglobulin light chain variable (V) region genes influence clinical presentation and outcome in light chain-associated amyloidosis (AL). *Blood*, 101, 3801–8.

Almeida, M. R. and Saraiva, M. J. (2012). Clearance of extracellular misfolded proteins in systemic amyloidosis: experience with transthyretin. *FEBS Lett*, 586, 2891–6.

Ancsin, J. B. (2003). Amyloidogenesis: historical and modern observations point to heparan sulfate proteoglycans as a major culprit. *Amyloid*, 10, 67–79.

Andersson, K., Olofsson, A., Nielsen, E. H., *et al.* (2002). Only amyloidogenic intermediates of transthyretin induce apoptosis. *Biochem Biophys Res Comm*, 294, 309–14.

Arbustini, E., Morbini, P., Verga, L., *et al.* (1997). Light and electron microscopy immunohistochemical characterization of amyloid deposits. *Amyloid*, 4, 157–70.

Ballardie, F. W., Kerr, D. N. S., Tennent, G., *et al.* (1986). Haemodialysis versus CAPD: equal predisposition to amyloidosis? *Lancet*, i, 795–6.

Bardin, T., Zingraff, J., Kuntz, D., *et al.* (1986). Dialysis-related amyloidosis. *Nephrol Dial Transplant*, 1, 151–4.

Benson, M. D. (2005). Ostertag revisited: the inherited systemic amyloidoses without neuropathy. *Amyloid*, 12, 75–87.

Benson, M. D., Kluve-Beckerman, B., Zeldenrust, S. R., *et al.* (2006). Targeted suppression of an amyloidogenic transthyretin with antisense oligonucleotides. *Muscle Nerve*, 33, 609–18.

Bergesio, F., Ciciani, A. M., Manganaro, M., *et al.* (2008). Renal involvement in systemic amyloidosis: an Italian collaborative study on survival and renal outcome. *Nephrol Dial Transplant*, 23, 941–51.

Berglund, K., Thysell, H., and Keller, C. (1993). Results, principles and pitfalls in the management of renal AA-amyloidosis; a 10–21 year followup of 16 patients with rheumatic disease treated with alkylating cytostatics. *J Rheumatol*, 20, 2051–7.

Bodin, K., Ellmerich, S., Kahan, M. C., *et al.* (2010). Antibodies to human serum amyloid P component eliminate visceral amyloid deposits. *Nature*, 468, 93–7.

Brenner, D. A., Jain, M., Pimentel, D. R., *et al.* (2004). Human amyloidogenic light chains directly impair cardiomyocyte function through an increase in cellular oxidant stress. *Circ Res*, 94, 1008–10.

Campioni, S., Mannini, B., Zampagni, M., *et al.* (2010). A causative link between the structure of aberrant protein oligomers and their toxicity. *Nat Chem Biol*, 6, 140–7.

Chiti, F. and Dobson, C. M. (2006). Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem*, 75, 333–66.

Coelho, T., Maia, L. F., Martins Da Silva, A., *et al.* (2012). Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. *Neurology*, 79, 785–92.

Cohen, A. S. and Calkins, E. (1959). Electron microscopic observations on a fibrous component in amyloid of diverse origins. *Nature*, 183, 1202–3.

Comenzo, R. L., Zhang, Y., Martinez, C., *et al.* (2001). The tropism of organ involvement in primary systemic amyloidosis: contributions of Ig V-L germ line gene use and clonal plasma cell burden. *Blood*, 98, 714–20.

De Beer, F. C., Mallya, R. K., Fagan, E. A., *et al.* (1982). Serum amyloid A protein (SAA) concentration in inflammatory diseases and its relationship to the incidence of reactive systemic amyloidosis. *Lancet*, ii, 231–4.

Dember, L. M. (2006). Amyloidosis-associated kidney disease. *J Am Soc Nephrol*, 17, 3458–71.

Dember, L. M., Hawkins, P. N., Hazenberg, B. P., *et al.* (2007). Eprodisate for the treatment of renal disease in AA amyloidosis. *N Engl J Med*, 356, 2349–60.

Dember, L. M., Sanchorawala, V., Seldin, D. C., *et al.* (2001). Effect of dose-intensive intravenous melphalan and autologous blood stem-cell transplantation on AL amyloidosis-associated renal disease. *Ann Med*, 134, 746–53.

Dey, B. R., Chung, S. S., Spitzer, T. R., *et al.* (2010). Cardiac transplantation followed by dose-intensive melphalan and autologous stem-cell transplantation for light chain amyloidosis and heart failure. *Transplantation*, 90, 905–11.

Dispenzieri, A., Gertz, M. A., Kyle, R. A., *et al.* (2004). Serum cardiac troponins and N-terminal pro-brain natriuretic peptide: a staging system for primary systemic amyloidosis. *J Clin Oncol*, 22, 3751–7.

Dispenzieri, A., Kyle, R. A., Gertz, M. A., *et al.* (2003). Survival in patients with primary systemic amyloidosis and raised serum cardiac troponins. *Lancet*, 361, 1787–9.

Drueke, T. B. and Massy, Z. A. (2009). Beta2-microglobulin. *Semin Dial*, 22, 378–80.

Dubrey, S. W., Burke, M. M., Khaghani, A., *et al.* (2001). Long term results of heart transplantation in patients with amyloid heart disease. *Heart*, 85, 202–7.

Eanes, E. D. and Glenner, G. G. (1968). X-ray diffraction studies on amyloid filaments. *J Histochem Cytochem*, 16, 673–7.

Elimova, E., Kisilevsky, R., and Ancsin, J. B. (2009). Heparan sulfate promotes the aggregation of HDL-associated serum amyloid A: evidence for a proamyloidogenic histidine molecular switch. *FASEB J*, 23, 3436–48.

Emiroglu, R., Basaran, O., Pehlivan, S., *et al.* (2005). Effect of amyloidosis on long-term survival in kidney transplantation. *Transplant Proc*, 37, 2967–8.

Falk, R. H. (2005). Diagnosis and management of the cardiac amyloidoses. *Circulation*, 112, 2047–60.

Filipowicz-Sosnowska, A. M., Roztropowicz-Denisiewicz, K., Rosenthal, C. J., *et al.* (1978). The amyloidosis of juvenile rheumatoid arthritis—comparative studies in Polish and American children. I. Levels of serum SAA protein. *Arthritis Rheum*, 21, 699–703.

Fish, R., Pinney, J., Jain, P., *et al.* (2010). The incidence of major hemorrhagic complications after renal biopsies in patients with monoclonal gammopathies. *Clin J Am Soc Nephrol*, 5(11), 1977–80.

Gejyo, F., Amano, I., Ando, T., *et al.* (2013). Survey of the effects of a column for adsorption of beta2-microglobulin in patients with dialysis-related amyloidosis in Japan. *Ther Apher Dial*, 17, 40–7.

Gertz, M. A., Lacy, M. Q., Dispenzieri, A., *et al.* (2007). Effect of hematologic response on outcome of patients undergoing transplantation for primary amyloidosis: importance of achieving a complete response. *Haematologica*, 92, 1415–18.

Gertz, M. A., Leung, N., Lacy, M. Q., *et al.* (2009). Clinical outcome of immunoglobulin light chain amyloidosis affecting the kidney. *Nephrol Dial Transplant*, 24, 3132–7.

Gillmore, J. D., Lachmann, H. J., Rowczenio, D., *et al.* (2009). Diagnosis, pathogenesis, treatment, and prognosis of hereditary fibrinogen A alpha-chain amyloidosis. *J Am Soc Nephrol*, 20, 444–51.

Gillmore, J. D., Madhoo, S., Pepys, M. B., *et al.* (2000). Renal transplantation for amyloid end-stage renal failure—insights from serial serum amyloid P component scintigraphy. *Nucl Med Comm*, 21, 735–40.

Gillmore, J. D., Stangou, A. J., Lachmann, H. J., *et al.* (2006). Organ transplantation in hereditary apolipoprotein AI amyloidosis. *Am J Transplant*, 6, 2342–7.

Greenwald, J. and Riek, R. (2010). Biology of amyloid: structure, function, and regulation. *Structure*, 18, 1244–60.

Gregorini, G., Izzi, C., Obici, L., *et al.* (2005). Renal apolipoprotein A-I amyloidosis: a rare and usually ignored cause of hereditary tubulointerstitial nephritis. *J Am Soc Nephrol*, 16, 3680–6.

Hartley, D. M., Walsh, D. M., Ye, C. P., *et al.* (1999). Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *J Neurosci*, 19, 8876–84.

Hawkins, P. N., Myers, M. J., Lavender, J. P., *et al.* (1988). Diagnostic radionuclide imaging of amyloid: biological targeting by circulating human serum amyloid P component. *Lancet*, 1, 1413–8.

Hawkins, P. N. and Pepys, M. B. (1990). A primed state exists in vivo following histological regression of amyloidosis. *Clin Exp Immunol*, 81, 325–8.

Immonen, K., Finne, P., Hakala, M., *et al.* (2008). No improvement in survival of patients with amyloidosis associated with inflammatory rheumatic diseases—data from the Finnish national registry for kidney diseases. *J Rheumatol*, 35, 1334–8.

Jacob, E. T., Bar-Nathan, N., Shapira, Z., *et al.* (1979). Renal transplantation in the amyloidosis of familial Mediterranean fever. Experience of ten cases. *Arch Intern Med*, 139, 1135–8.

Jadoul, M. (1998). Dialysis-related amyloidosis: importance of biocompatibility and age. *Nephrol Dial Transplant*, 13 Suppl, 61–4.

Keeling, J. and Herrera, G. A. (2005). Matrix metalloproteinases and mesangial remodeling in light chain-related glomerular damage. *Kidney Int*, 68, 1590–603.

Kluve-Beckerman, B., Hardwick, J., Du, L., *et al.* (2011). Antisense oligonucleotide suppression of serum amyloid A reduces amyloid deposition in mice with AA amyloidosis. *Amyloid*, 18(3), 136–46.

Klyubin, I., Walsh, D. M., Lemere, C. A., *et al.* (2005). Amyloid beta protein immunotherapy neutralizes Abeta oligomers that disrupt synaptic plasticity in vivo. *Nat Med*, 11, 556–61.

Kristen, A. V., Dengler, T. J., Hegenbart, U., *et al.* (2008). Prophylactic implantation of cardioverter-defibrillator in patients with severe cardiac amyloidosis and high risk for sudden cardiac death. *Heart Rhythm*, 5, 235–40.

Kyle, R. A. and Gertz, M. A. (1995). Primary systemic amyloidosis: clinical and laboratory features in 474 cases. *Semin Hematol* 32, 45–59.

Kyle, R. A., Therneau, T. M., Rajkumar, S. V., *et al.* (2002). A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *N Engl J Med*, 346, 564–9.

Lachmann, H. J., Gallimore, R., Gillmore, J. D., *et al.* (2003). Outcome in systemic AL amyloidosis in relation to changes in concentration of circulating free immunoglobulin light chains following chemotherapy. *Br J Haematol*, 122, 78–84.

Lachmann, H. J., Gillmore, J. D., Wechalekar, A. D., *et al.* (2010). Survival on dialysis and outcome after renal transplantation in AA amyloidosis. *Amyloid*, 17 (Suppl. 1), 73.

Lachmann, H. J., Goodman, H. J. B., Gilbertson, J. A., *et al.* (2007). Natural history and outcome in systemic AA amyloidosis. *N Engl J Med*, 356, 2361–71.

Laiho, K., Tiitinen, S., Kaarela, K., *et al.* (1999). Secondary amyloidosis has decreased in patients with inflammatory joint disease in Finland. *Clin Rheumatol*, 18, 122–3.

Lambert, M. P., Barlow, A. K., Chromy, B. A., *et al.* (1998). Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci U S A*, 95, 6448–53.

Lavatelli, F., Perlman, D. H., Spencer, B., *et al.* (2008). Amyloidogenic and associated proteins in systemic amyloidosis proteome of adipose tissue. *Mol Cell Proteomics*, 7, 1570–83.

Leung, N., Glavey, S. V., Kumar, S., *et al.* (2013). A detailed evaluation of the current renal response criteria in AL amyloidosis: is it time for a revision? *Haematologica*, 98, 988–92.

Leung, N., Griffin, M. D., Dispenzieri, A., *et al.* (2005). Living donor kidney and autologous stem cell transplantation for primary systemic amyloidosis (AL) with predominant renal involvement. *Am J Transplant*, 5, 1660–70.

Li, J. P., Galvis, M. L., Gong, F., *et al.* (2005). In vivo fragmentation of heparan sulfate by heparanase overexpression renders mice resistant to amyloid protein A amyloidosis. *Proc Natl Acad Sci U S A*, 102, 6473–7.

Liao, R. L., Jain, M., Teller, P., *et al.* (2001). Infusion of light chains from patients with cardiac amyloidosis causes diastolic dysfunction in isolated mouse hearts. *Circulation*, 104, 1594–7.

Lobato, L., Ventura, A., Beirão, I., *et al.* (2003). End-stage renal disease in familial amyloidosis ATTR Val30Met: a definitive indication to combined liver-kidney transplantation *Transplant Proc*, 35, 1116–20.

Lovat, L. B., Persey, M. R., Madhoo, S., *et al.* (1998). The liver in systemic amyloidosis: insights from ^{123}I serum amyloid P component scintigraphy in 484 patients. *Gut*, 42, 727–34.

Maceira, A. M., Joshi, J., Prasad, S. K., *et al.* (2005). Cardiovascular magnetic resonance in cardiac amyloidosis. *Circulation*, 111, 186–93.
Martin, D. J. and Ramirez-Alvarado, M. (2011). Glycosaminoglycans promote fibril formation by amyloidogenic immunoglobulin light chains through a transient interaction. *Biophys Chem*, 158, 81–9.
Maurer, M. S., Raina, A., Hesdorffer, C., *et al.* (2007). Cardiac transplantation using extended-donor criteria organs for systemic amyloidosis complicated by heart failure. *Transplantation*, 83, 539–45.
Merlini, G. (2012). CyBorD: stellar response rates in AL amyloidosis. *Blood*, 119, 4343–5.
Merlini, G., Ascari, E., Amboldi, N., *et al.* (1995). Interaction of the anthracycline 4'-iodo-4'-deoxydoxorubicin with amyloid fibrils: inhibition of amyloidogenesis. *Proc Natl Acad Sci U S A*, 92, 2959–63.
Merlini, G. and Bellotti, V. (2003). Molecular mechanisms of amyloidosis. *N Engl J Med*, 349, 583–96.
Merlini, G., Seldin, D. C., and Gertz, M. A. (2011). Amyloidosis: pathogenesis and new therapeutic options. *J Clin Oncol*, 29, 1924–33.
Merlini, G. and Stone, M. J. (2006). Dangerous small B-cell clones. *Blood*, 108, 2520–30.
Merlini, G., Wechalekar, A. D., and Palladini, G. (2013). Systemic light chain amyloidosis: an update for treating physicians. *Blood*, 121(26), 5124–30.
Mesquita, M., Fosso, C., Bakoto Sol, E., *et al.* (2011). Renal biopsy findings in Belgium: a retrospective single center analysis. *Acta Clinica Belgica* 66, 104–9.
Miyata, T., Jadoul, M., Kurokawa, K., *et al.* (1998). Beta-2 microglobulin in renal disease. *J Am Soc Nephrol*, 9, 1723–35.
Montagna, G., Cazzulani, B., Obici, L., *et al.* (2013). Benefit of doxycycline treatment on articular disability caused by dialysis related amyloidosis. *Amyloid*, 20(3), 173–8.
Motamedi-Shad, N., Monsellier, E., Torrassa, S., *et al.* (2009). Kinetic analysis of amyloid formation in the presence of heparan sulfate: faster unfolding and change of pathway. *J Biol Chem*, 284, 29921–34.
Murphy, C. L., Wang, S., Kestler, D., *et al.* (2010). Leukocyte chemotactic factor 2 (LECT2)-associated renal amyloidosis: a case series. *Am J Kidney Dis*, 56, 1100–7.
Myllykangas-Luosujärvi, R., Aho, K., Kautiainen, H., *et al.* (1999). Amyloidosis in a nationwide series of 1666 subjects with rheumatoid arthritis who died during 1989 in Finland. *Rheumatology*, 38, 499–503.
Noborn, F., O'callaghan, P., Hermansson, E., *et al.* (2011). Heparan sulfate/heparin promotes transthyretin fibrillization through selective binding to a basic motif in the protein. *Proc Natl Acad Sci U S A*, 108, 5584–9.
Obici, L., Cortese, A., Lozza, A., *et al.* (2012). Doxycycline plus tauroursodeoxycholic acid for transthyretin amyloidosis: a phase II study. *Amyloid*, 19 Suppl 1, 34–6.
Obici, L., Palladini, G., Giorgetti, S., *et al.* (2004). Liver biopsy discloses a new apolipoprotein A-I hereditary amyloidosis in several unrelated Italian families. *Gastroenterology*, 126, 1416–22.
Obici, L., Perfetti, V., Palladini, G., *et al.* (2005). Clinical aspects of systemic amyloid diseases. *Biochim Biophys Acta*, 1753, 11–22.
Obici, L., Raimondi, S., Lavatelli, F., *et al.* (2009). Susceptibility to AA amyloidosis in rheumatic diseases: a critical overview. *Arthritis Rheum*, 61, 1435–40.
Palladini, G., Campana, C., Klersy, C., *et al.* (2003). Serum N-terminal pro-brain natriuretic peptide is a sensitive marker of myocardial dysfunction in AL amyloidosis. *Circulation*, 107, 2440–5.
Palladini, G., Dispenzieri, A., Gertz, M. A., *et al.* (2012). New criteria for response to treatment in immunoglobulin light chain amyloidosis based on free light chain measurement and cardiac biomarkers: impact on survival outcomes. *J Clin Oncol*, 30, 4541–9.
Palladini, G., Perfetti, V., Obici, L., *et al.* (2004). Association of melphalan and high-dose dexamethasone is effective and well tolerated in patients with AL (primary) amyloidosis who are ineligible for stem cell transplantation. *Blood*, 103, 2936–8.
Pepys, M. B. (2006). Amyloidosis. *Annu Rev Med*, 57, 223–41.
Pepys, M. B., Booth, D. R., Hutchinson, W. L., *et al.* (1997). Amyloid P component. A critical review. *Amyloid*, 4, 274–95.
Pepys, M. B., Herbert, J., Hutchinson, W. L., *et al.* (2002). Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. *Nature*, 417, 254–9.
Perfetti, V., Casarini, S., Palladini, G., *et al.* (2002). Analysis of V lambda-J lambda expression in plasma cells from primary (AL) amyloidosis and normal bone marrow identifies 3r (lambda III) as a new amyloid-associated germline gene segment. *Blood*, 100, 948–53.
Perfetti, V., Colli Vinarelli, M., Anesi, E., *et al.* (1999). The degrees of plasma cell clonality and marrow infiltration adversely influence the prognosis of AL amyloidosis patients. *Haematologica*, 84, 218–21.
Perfetti, V., Palladini, G., Casarini, S., *et al.* (2012). The repertoire of lambda light chains causing predominant amyloid heart involvement and identification of a preferentially involved germline gene, IGLV1-44. *Blood*, 119, 144–50.
Phipps, J. E., Kestler, D. P., Foster, J. S., *et al.* (2010). Inhibition of pathologic immunoglobulin-free light chain production by small interfering RNA molecules. *Exp Hematol*, 38, 1006–13.
Pinney, J. H., Lachmann, H. J., Bansi, L., *et al.* (2011). Outcome in renal AL amyloidosis following chemotherapy. *J Clin Oncol*, 29, 674–81.
Rajkumar, S. V., Gertz, M. A., and Kyle, R. A. (1998). Primary systemic amyloidosis with delayed progression to multiple myeloma. *Cancer*, 82, 1501–5.
Rapezzi, C., Quarta, C. C., Guidalotti, P. L., *et al.* (2011). Usefulness and limitations of 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy in the aetiological diagnosis of amyloidotic cardiomyopathy. *Eur J Nucl Med Mol Imaging*, 38, 470–8.
Relini, A., Canale, C., De Stefano, S., *et al.* (2006). Collagen plays an active role in the aggregation of beta 2-microglobulin under physio-pathological conditions of dialysis-related amyloidosis. *J Biol Chem*, 281(24), 16521–9.
Relini, A., De Stefano, S., Torrassa, S., *et al.* (2008). Heparin strongly enhances the formation of beta2-microglobulin amyloid fibrils in the presence of type I collagen. *J Biol Chem*, 283, 4912–20.
Ren, R., Hong, Z., Gong, H., *et al.* (2010). Role of glycosaminoglycan sulfation in the formation of immunoglobulin light chain amyloid oligomers and fibrils. *J Biol Chem*, 285, 37672–82.
Rocken, C., Schwotzer, E. B., Linke, R. P., *et al.* (1996). The classification of amyloid deposits in clinicopathological practice. *Histopathology*, 29, 325–35.
Said, S. M., Sethi, S., Valeri, A. M., *et al.* (2013). Renal amyloidosis: origin and clinicopathologic correlations of 474 recent cases. *Clin J Am Soc Nephrol*, 8(9), 1515–23.
Saraiva, M. J. (2002). Hereditary transthyretin amyloidosis: molecular basis and therapeutical strategies. *Expert Rev Mol Med* 4, 1–11.
Sattianayagam, P., Gibbs, S. D. J., Wechalekar, A. D., *et al.* (2009). Solid organ transplantation in AL amyloidosis. *Amyloid*, 17, 149.
Schenk, D., Games, D., and Seubert, P. (2001). Potential treatment opportunities for Alzheimer's disease through inhibition of secretases and A beta immunization. *J Mol Neurosci*, 17, 259–67.
Schiffl, H. (2014). Impact of advanced dialysis technology on the prevalence of dialysis-related amyloidosis in long-term maintenance dialysis patients. *Hemodial Int*, 18(1), 136–41.
Schnitzer, T. J. and Ansell, B. M. (1977). Amyloidosis in juvenile chronic polyarthritis. *Arthritis Rheum*, 20, 245–52.
Schwalbe, S., Holzhauer, M., Schaeffer, J., *et al.* (1997). Beta 2-microglobulin associated amyloidosis: a vanishing complication of long-term hemodialysis? *Kidney Int*, 52, 1077–83.
Sethi, S., Theis, J. D., Vrana, J. A., *et al.* (2013). Laser microdissection and proteomic analysis of amyloidosis, cryoglobulinemic GN, fibrillary GN, and immunotactoid glomerulopathy. *Clin J Am Soc Nephrol*, 8, 915–21.
Sherif, A. M., Refaie, A. F., Sobh, M. A., *et al.* (2003). Long-term outcome of live donor kidney transplantation for renal amyloidosis. *Am J Kidney Dis*, 42, 370–5.
Shi, J., Guan, J., Jiang, B., *et al.* (2010). Amyloidogenic light chains induce cardiomyocyte contractile dysfunction and apoptosis via a

non-canonical p38alpha MAPK pathway. *Proc Natl Acad Sci U S A*, 107, 4188–93.

Silveira, J. R., Raymond, G. J., Hughson, A. G., *et al.* (2005). The most infectious prion protein particles. *Nature*, 437, 257–61.

Solomon, A., Weiss, D. T., and Wall, J. S. (2003). Immunotherapy in systemic primary (AL) amyloidosis using amyloid-reactive monoclonal antibodies. *Cancer Biother Radiopharmaceut*, 18, 853–60.

Soni, A. and Lelorier, P. (2005). Sudden death in nondilated cardiomyopathies: pathophysiology and prevention. *Curr Heart Fail Rep*, 2, 118–23.

Sousa, M. M., Cardoso, I., Fernandes, R., *et al.* (2001). Deposition of transthyretin in early stages of familial amyloidotic polyneuropathy—Evidence for toxicity of nonfibrillar aggregates. *Am J Pathol*, 159, 1993–2000.

Specter, R., Sanchorawala, V., Seldin, D. C., *et al.* (2011). Kidney dysfunction during lenalidomide treatment for AL amyloidosis. *Nephrol Dial Transplant*, 26, 881–6.

Stangou, A. J., Banner, N. R., Hendry, B. M., *et al.* (2010). Hereditary fibrinogen A alpha-chain amyloidosis: phenotypic characterization of a systemic disease and the role of liver transplantation. *Blood*, 115, 2998–3007.

Svantesson, H., Akesson, A., Eberhardt, K., *et al.* (1983). Prognosis in juvenile rheumatoid arthritis with systemic onset. A follow-up study. *Scand J Rheumatol*, 12, 139–44.

Swiecicki, P. L., Edwards, B. S., Kushwaha, S. S., *et al.* (2013). Left ventricular device implantation for advanced cardiac amyloidosis. *J Heart Lung Transplant*, 32, 563–8.

Tan, S. Y., Irish, A., Winearls, C. G., *et al.* (1996). Long term effect of renal transplantation on dialysis-related amyloid deposits and symptomatology. *Kidney Int*, 50, 282–9.

Tang, W., Mcdonald, S. P., Hawley, C. M., *et al.* (2013). End-stage renal failure due to amyloidosis: outcomes in 490 ANZDATA registry cases. *Nephrol Dial Transplant*, 28, 455–61.

Teng, J., Russell, W. J., Gu, X., *et al.* (2004). Different types of glomerulopathic light chains interact with mesangial cells using a common receptor but exhibit different intracellular trafficking patterns. *Lab Invest*, 84, 440–51.

Traut, M., Haufe, C. C., Eismann, U., *et al.* (2007). Increased binding of beta-2-microglobulin to blood cells in dialysis patients treated with high-flux dialyzers compared with low-flux membranes contributed to reduced beta-2-microglobulin concentrations. Results of a cross-over study. *Blood Purif*, 25, 432–40.

Tufveson, G., Geerlings, W., Brunner, F. P., *et al.* (1989). The combined report on regular dialysis and transplantation in Europe. XIX 1988. *Nephrol Dial Transplant*, 4, 1–30.

Van Gameren, I., Hazenberg, B. P., Bijzet, J., *et al.* (2006). Diagnostic accuracy of subcutaneous abdominal fat tissue aspiration for detecting systemic amyloidosis and its utility in clinical practice. *Arthritis Rheum*, 54, 2015–21.

Vrana, J. A., Gamez, J. D., Madden, B. J., *et al.* (2009). Classification of amyloidosis by laser microdissection and mass spectrometry-based proteomic analysis in clinical biopsy specimens. *Blood*, 114, 4957–9.

Wall, J. S., Kennel, S. J., Williams, A., *et al.* (2012). AL amyloid imaging and therapy with a monoclonal antibody to a cryptic epitope on amyloid fibrils. *PLoS One*, 7, e52686.

Wall, J. S., Richey, T., Stuckey, A., *et al.* (2011). In vivo molecular imaging of peripheral amyloidosis using heparin-binding peptides. *Proc Natl Acad Sci U S A*, 108(34), E586–94.

Walsh, D. M., Klyubin, I., Fadeeva, J. V., *et al.* (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature*, 416, 535–9.

Wechalekar, A. D., Lachmann, H. J., Goodman, H. J., *et al.* (2008). AL amyloidosis associated with IgM paraproteinemia: clinical profile and treatment outcome. *Blood*, 112, 4009–16.

Witzig, T. E., Timm, M., Larson, D., *et al.* (1999). Measurement of apoptosis and proliferation of bone marrow plasma cells in patients with plasma cell proliferative disorders. *Br J Haematol*, 104, 131–7.

Yamamoto, S. and Gejyo, F. (2005). Historical background and clinical treatment of dialysis-related amyloidosis. *Biochim Biophys Acta*, 1753, 4–10.

CHAPTER 153

The patient with myeloma

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Introduction

Renal impairment is a common feature of symptomatic multiple myeloma (MM). It is not rare for a patient with myeloma to present to the nephrology clinic for evaluation of renal impairment, which is usually of recent onset, or to be hospitalized because of acute kidney injury (AKI). Conversely, in a patient with MM, development of RI may provide a clue to the diagnosis of the disease and may cause major management problems.

Depending on the definition of renal failure this complication occurs in 20–40% of newly diagnosed patients with MM (Alexanian et al., 1990; Bladé et al., 1998; Kyle et al., 2003; Eleutherakis-Papaiakovou et al., 2007). When a cut-off of serum creatinine level > 2 mg/dL is used, about 20% of patients with MM have renal failure at diagnosis (Alexanian et al., 1990; Bladé et al., 1998; Knudsen et al., 2000). When renal failure is defined by a serum creatinine > 1.5 mg/dL, the frequency increases up to 30% or even 50% in some series (Knudsen et al., 2000). When the definition includes those with an estimated glomerular filtration rate (eGFR) < 50 mL/min/1.73 m^2 then even higher proportions of patients with MM present with moderate to severe renal dysfunction. Most patients present with moderate renal impairment and serum creatinine levels are <4 mg/dL; however, in series from tertiary hospitals up to 10% of patients with newly diagnosed MM have renal failure severe enough to require renal replacement with dialysis at the time of diagnosis (Torra et al., 1995). It has also been considered that an additional 25% of patients develop renal failure later in the course of their disease. Careful assessment of the renal function is needed during initial evaluation of a patient with newly diagnosed myeloma in order to establish whether a potentially nephrotoxic light chain may exist that in future relapse may cause significant renal damage. In several cases with late-onset renal failure, hypercalcaemia may be the cause of renal dysfunction, which due to the use of bisphosphonates to treat myeloma bone disease is becoming much less frequent. Other causes of late-onset renal dysfunction may also include potentially nephrotoxic drugs that are used to treat complications of myeloma (e.g. bisphosphonates), non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics.

In the mid 1940s, it was recognized that renal failure in patients with myeloma was associated with the excretion of Bence Jones proteins and factors that could modulate their concentration or urine pH may be important in the development of myeloma kidney. Often, acute RI was precipitated after an intravenous urography (Bartels et al., 1954; Brodwall et al., 1956; Killmann et al., 1957; Perillie and Conn, 1958; Healy, 1963; Vix, 1966) and the use of this diagnostic method was contraindicated in patients with myeloma. The first attempts to manage myeloma-related acute AKI were based on simple measures such as vigorous hydration and limited protein-containing diet and dialysis, when this became available, mainly due to the lack of an effective antimyeloma therapy (Blackman et al., 1944; Meachem and Heinle, 1950; Anonymous, 1959). However, the clinical course of most patients presenting with myeloma-related renal impairment was poor, and often patients died within a few weeks or months (Blackman et al., 1944; Malpas, 1969).

Pathogenesis of myeloma-related acute renal damage

Renal failure in patients with MM results from the toxic effects of the monoclonal light chains to the renal structures, mainly renal tubules and less often to glomeruli. Hypercalcaemia is a less common cause of renal insufficiency and is usually associated with toxic light chains. Other factors that contribute to or exacerbate renal impairment include dehydration, nephrotoxic drugs (antibiotics, NSAIDs), and perhaps contrast agents. Usually these factors aggravate the toxic effects of light chains and are rarely the primary reason of renal failure.

Monoclonal light chains cause renal damage by distinct mechanisms and in various segments of the nephron, glomeruli, tubules, interstitium, and blood vessels, thus resulting in different pathologic and clinical findings. By far the most frequent form of renal damage is myeloma cast nephropathy (the so-called myeloma kidney). Other clinicopathological conditions associated with the effects of light chains in the kidney include amyloidosis, monoclonal immunoglobulin deposition disease (MIDD), most often of light chains (light chain deposition disease (LCDD)), or acquired adult Fanconi syndrome. The effect of monoclonal immunoglobulins in the kidneys may include a variety of pathologic conditions, but usually are not associated with overt myeloma (Leung et al., 2012). Furthermore, these pathologic entities may sometimes coexist in the same patient so that in a patient with myeloma presenting with RI a renal biopsy may show cast nephropathy along with amyloid or amorphous deposits in the glomeruli.

Pathobiology of the light chains

Under normal conditions the circulating monoclonal light chains are relatively freely filtered through the glomerulus and reach the proximal tubule where they are catabolized. Free light chains (FLCs) are endocytosed by proximal tubule cells through a receptor mediated process: they bind to the tandem scavenger receptor system of cubilin/megalin and then they are endocytosed via the clathrin-dependent endosomal/lysosomal pathway and degraded

within lysosomes (Batuman et al., 1990, 1998; Batuman and Guan, 1997; Santostefano et al., 2005) (Fig. 153.1). In MM patients, the excess light chain production overcomes the capacity of the tubular cells to endocytose and catabolize the FLCs which thus appear in increased amounts in the tubular fluid of distal nephron segments where they form tubular casts with Tamm–Horsfall protein (uromodulin), a glycoprotein synthesized by the cells in the medullary thick ascending limb of the loop of Henle. Light chains interact through their complementary determining region (CDR) with a specific binding site on the Tamm–Horsfall protein and may form aggregates and casts that subsequently lead to tubular obstruction of the distal tubule and of the thick ascending loop of Henle (Huang and Sanders, 1995, 1997; Ying and Sanders, 2001) (Fig. 153.1). Factors such as dehydration, hypercalcaemia, acidosis, and furosemide promote the formation of aggregates of light chain/Tamm–Horsfall protein (Pirani et al., 1987; Sanders et al., 1990; Sanders and Booker, 1992; Ying and Sanders, 2001). The resulting tubular obstruction increases intraluminal pressure, reduces glomerular filtration rate (GFR), and reduces interstitial blood flow, thus further compromising the renal function. The reduced tubular clearance of light chains further increases their concentration in the tubules and contributes to the vicious circle that results in myeloma cast nephropathy. The probability of cast formation increases when the amount of light chains increase, however, there is considerable diversity among the nephrotoxicity of light chains: some patients may have significant renal damage with only small amounts of light chains while in others even large amounts (in the range of several grams of light chains per day) cause minimal or no dysfunction. This is largely related to the affinity of the light chain for Tamm–Horsfall protein. Of note, when a light chain is nephrotoxic it usually causes renal dysfunction early in the course of the disease, even before other clinical manifestations of MM become apparent (Bladé and Rosinol, 2005). As expected, different light chains also differ significantly to the type of renal damage that they cause. In general, the variable region of the light chain (V_L) determines nephrotoxicity of the specific light chain by determining, for example, the affinity with Tamm–Horsfall protein (Sanders et al., 1990; Sanders and Booker, 1992). It has been suggested that Tamm–Horsfall protein interacts with the hypervariable regions of the light chains. This region contains the amino acids that give diversity, conformation flexibility, and allow for interactions with various proteins to promote antigen binding by immunoglobulins (Solomon et al., 1991; Batuman, 2007). Furthermore, the V_L region probably determines the specific type of renal damage that a light chain can cause. Both lambda and kappa light chains are nephrotoxic but lambda light chains are more frequently involved in the formation of amyloid than kappa (Comenzo et al., 2001) and kappa are more frequently involved in the other types of renal damage such as LCDD (Ronco et al., 2006) and acquired adult Fanconi syndrome (Ma et al., 2004).

Cast formation is not the only pathophysiologic mechanism by which the light chains cause renal damage in myeloma kidney. Endocytosis of light chain by renal tubular cells also induces pro-inflammatory cytokine production, such as interleukin-6, -8, and tumour necrosis factor alpha, by these cells, mainly mediated through activation of nuclear factor kappa B (NF-κB) and mitogen activated protein kinases (Sengul et al., 2002, 2003). Recently it has been shown that Src kinase-dependent activation of the NF-κB pathway increases the production of monocyte chemoattractant protein-1 (MCP-1) (Ying et al., 2011). These proinflammatory cytokines promote infiltration by inflammatory cells that produce metalloproteinases and increase transforming growth factor beta production resulting in matrix protein deposition and subsequent fibrosis and further compromising the ability of the nephron to restore function (Keeling and Herrera, 2007). Light chains endocytosis may also cause tubular cell necrosis, leading to more severe renal dysfunction (Sanders et al., 1987). The exact mechanism has not been clarified but it has been suggested that aggregation of light chains after endocytosis may initiate a cascade resulting in tubular cell death. Light chains may also lead to functional impairment of tubular cells resulting in Fanconi syndrome (Ma et al., 2004). Focal loss of microvilli and inhibition of Na-K-ATPase may lead to reabsorption defects (Guan et al., 1999). Some myeloma patients also have a urine concentration defect, probably due to tubulointerstitial changes and nephrogenic diabetes insipidus due to unresponsiveness to antidiuretic hormone, thus further promoting dehydration (DeFronzo et al., 1978).

Glomerulopathy due to light chains is caused by the deposition of immunoglobulins either in the form of amyloid fibrils or amorphous deposits of non-amyloid structure. In both glomerulopathies, the dominant symptom is the development of non-selective proteinuria. The amyloid deposits are fibrillar structures that consist of the N-terminal fragments of the variable regions of light chains (Merlini and Bellotti, 2003). Amyloid deposits can be found in every portion of the kidney; however, they predominate within the glomeruli and give a positive Congo red staining. Glomerular depositions of amyloid usually present with significant proteinuria (often with daily urine protein excretion above 10–15 g) but renal failure is evident in no more than 20% of patients at diagnosis. However, 5–10% of patients with amyloidosis may have predominantly vascular rather than glomerular depositions and present with renal failure rather than nephrotic syndrome (Obici et al., 2005).

In LCDD, the light-chain deposits are non-fibrillar and Congo red staining is negative (Buxbaum and Gallo, 1999). Typically, granular depositions of light chains are observed within the mesangial areas, while thickening of the peripheral basement membrane may resemble type II membranoproliferative glomerulonephritis or diabetic lesions. These deposits may also be present in arterioles and capillaries. Diagnosis is supported by immunofluorescence (although in 10% of cases it may be negative), and by electron microscopy. Linear peritubular deposits of monotypic light chains are usually found but these deposits are also found along basement membrane, mesangial nodules, along Bowman's capsule, vascular structures, and in the interstitium. In addition to the glomerular findings, the presence of interstitial fibrosis is common (Pozzi et al., 2003). In early phases of the disease the glomerular lesions may be minimal and in this case the diagnostic suspicion comes from the finding of eosinophilic, periodic acid–Schiff-positive material, consisting of light chains, along the outer part of the tubular basement membrane. In contrast to amyloidosis, in which the light chain is of lambda type in 80% of cases (Obici et al., 2005), in LCDD the light chain is usually of kappa type (Pozzi et al., 2003). However, as in primary systemic amyloidosis, the characteristic clinical picture is of a nephrotic syndrome, but renal function is more severely and rapidly impaired than in amyloidosis: almost all patients with LCDD present with renal failure. Extrarenal involvement is less frequent than in amyloidosis.

Acquired Fanconi syndrome is an exceedingly unusual disorder characterized by failure in the reabsorptive capacity of the proximal

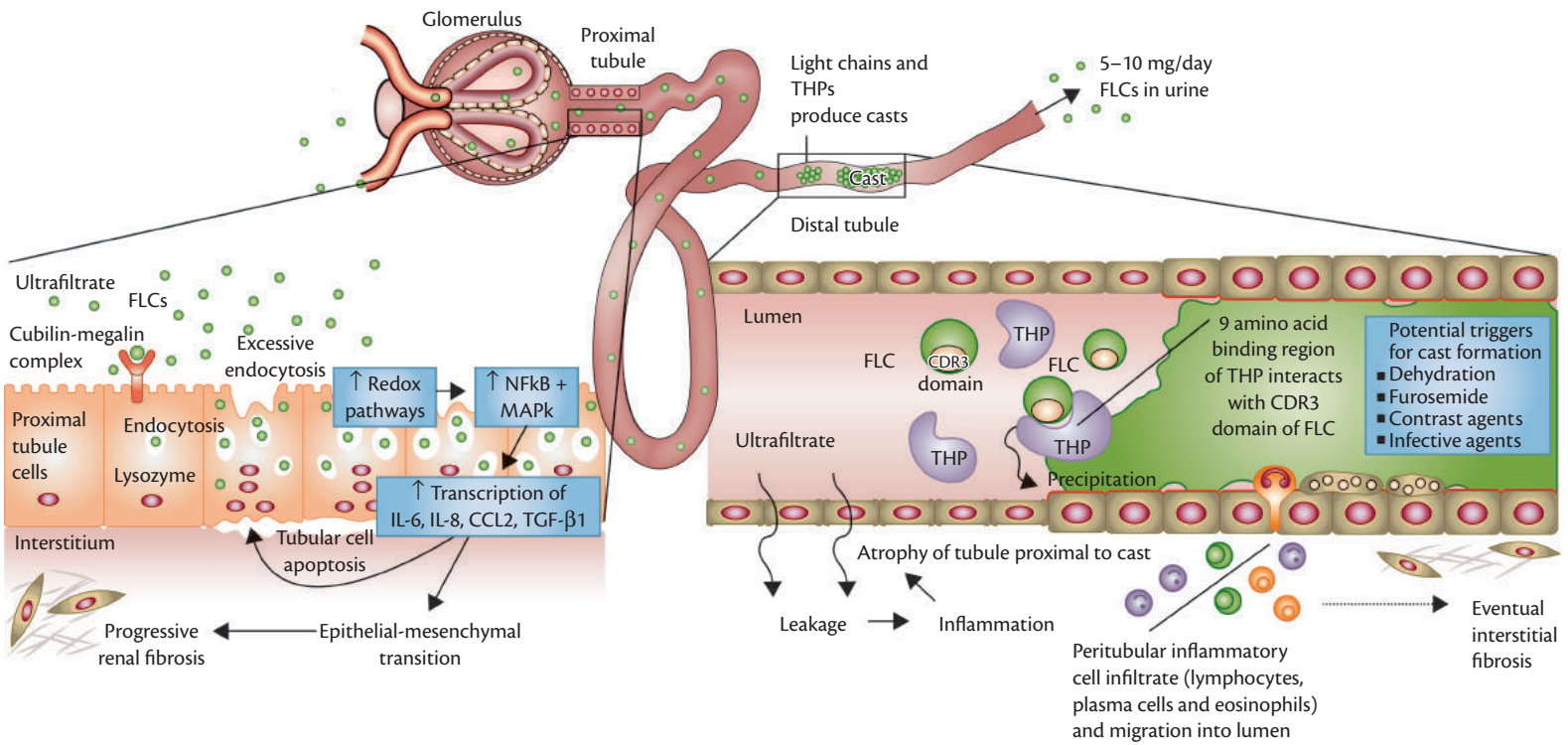

Fig. 153.1 Mechanisms of FLC-induced acute kidney injury. The very high concentrations of FLCs present in the ultrafiltrate of patients with multiple myeloma can result in direct injury to PTCs. The excessive endocytosis of FLCs by the cubilin–megalin complex expressed on PTCs can trigger apoptotic, proinflammatory and fibrotic pathways. Activation of redox pathways occurs, with increased expression of NFκB and MAPK, which in turn leads to the transcription of both inflammatory and profibrotic cytokines, such as IL-6, IL-8, CCL2, and TGF-β1. In the distal tubules, FLCs can bind to a specific binding domain on THPs and co-precipitate to form casts. These casts result in tubular atrophy proximal to the cast and lead to progressive interstitial inflammation and fibrosis. CCL2 = C-C motif chemokine 2; CDR = complementarity determining region; FLC = free light chain; IL = interleukin; MAPK = mitogen-activated protein kinase; NFκB = nuclear factor κB; PTC = proximal tubule cell; TGF-β1 = transforming growth factor beta 1; THP = Tamm–Horsfall protein.

renal tubules, resulting in glycosuria, aminoaciduria, hypophosphataemia, and hypouricaemia (Ma et al., 2004).The renal damage is caused by partially catabolized light chains that form crystalline inclusions within the proximal tubular cells interfering with membrane transporters. Kappa light chains are found in 90% of the cases. The most common clinical findings in patients with acquired Fanconi syndrome are bone pain from osteoporosis or slowly progressive renal insufficiency. Most patients are asymptomatic and the diagnosis usually comes from an unexplained hypouricaemia during the investigation of a patient with monoclonal gammopathy of undetermined significance—only rarely is this complication found in a patient with symptomatic MM.

Diagnosis and assessment of the patient with myeloma who presents with renal impairment

The presentation of renal impairment, especially if it is acute or relatively recent, in a patient with a monoclonal gammopathy, should be evaluated promptly in order to determine whether the monoclonal gammopathy is the cause of the kidney dysfunction. Conversely, in a patient presenting with acute- or recent-onset renal impairment, evaluation for an underlying monoclonal gammopathy should be instituted. Renal dysfunction due to myeloma cast nephropathy is reversible in many, if not most, patients, if managed immediately with appropriate treatment and the physician should be alert for the diagnosis of myeloma in a patient with AKI.

The diagnosis of myeloma requires the demonstration of a monoclonal immunoglobulin and demonstration of infiltration of the bone marrow (with either a marrow aspirate or a bone marrow trephine biopsy). A monoclonal immunoglobulin (either a complete heavy-light chain immunoglobulin or a monoclonal light-chain only immunoglobulin) is present in 95–98% of patients with myeloma. However, if a patient has myeloma-related kidney dysfunction a monoclonal immunoglobulin is almost always present. Only a few cases of non-secretory myeloma with biopsy-proven renal dysfunction due to myeloma have been described—these patients had minimal amounts of very toxic light chains which may have escaped immunofixation; however, with the use of the serum free light chain assay these patients may have a measurable amount of FLC (either kappa or lambda). Thus, complete evaluation includes assessment of serum FLCs.

The most critical question in a patient with monoclonal gammopathy presenting with renal dysfunction is whether the kidney dysfunction is due to the underlying monoclonal gammopathy/myeloma or, whether it is pre-existing and unrelated to myeloma. This determination is especially important for patients with asymptomatic myeloma, which may fulfil the criteria of myeloma in their bone marrow but they do not present other criteria of symptomatic disease (such as lytic bone lesions, anaemia, hypercalcaemia, or renal dysfunction). In these particular patients, the determination of causality is important since the development of renal impairment related to monoclonal gammopathy or myeloma is an absolute indication for immediate initiation of chemotherapy. During recent years, there are increasing numbers of patients who are diagnosed with monoclonal gammopathies; most of them are of advanced age so other conditions are common.

A 24-hour urine collection for the assessment of the amount and type of proteinuria may be helpful. In patients with cast nephropathy, the glomerulus is unaffected and thus FLCs (Bence Jones protein) will predominate in the urine; these patients will usually have a large spike in the gamma region and the urine immunofixation will show free kappa or lambda light chains. In patients with conditions affecting the glomerulus, such as amyloidosis of immunoglobulin deposition, as well as in conditions such as diabetic nephropathy, the proteinuria is mostly non-selective, and albumin will predominate. In a recent report, 24-hour urine protein containing < 25% of albumin had a sensitivity of 0.98, specificity of 0.94, positive predictive value of 0.75, and negative predictive value of 0.99 for the diagnosis of cast nephropathy (Leung et al., 2012). Thus, a renal biopsy is probably not necessary in patients with a high probability of cast nephropathy, especially if overt myeloma is present.

When should a renal biopsy be performed in a patient with multiple myeloma?

In patients with clinical features of MM with or without renal failure in whom proteinuria mainly consists of light chains, a renal biopsy may not be necessary and the cause of renal impairment may be attributed to myeloma cast nephropathy (Leung et al., 2012). However, renal biopsy may provide significant prognostic information and may be especially helpful in patients who may have coexisting conditions (Hutchison et al., 2012a). In patients in whom the main finding is a nephrotic syndrome with or without renal failure, the first diagnostic possibility is an associated systemic amyloidosis, especially if additional systemic symptoms are indicative of multiple organ involvement (e.g. symptoms and signs of heart dysfunction, liver enlargement, or neuropathy) (Merlini and Stone, 2006; Hutchison et al., 2012a; Merlini and Palladini, 2012). In this case, a renal biopsy may be the quickest and most informative test. A subcutaneous fat aspirate may be performed, which is safe but has sensitivity for the detection of systemic amyloidosis of only about 70%. If negative for amyloid a rectal biopsy may follow. If there is no demonstration of amyloid, the next step should be a kidney biopsy in search for amyloid, LCDD or an unrelated glomerulopathy, such as glomerulonephritis.

A renal biopsy in a patient with a monoclonal gammopathy and renal dysfunction may help to define the type of renal injury, assess the degree of activity of the pathological process which caused the RI, and assess whether a long-standing process may have caused irreversible damage, which in turn can influence the type of therapy and also provide valuable prognostic information regarding the renal outcome. In our practice we tend to refer our patients with myeloma or other monoclonal gammopathy for renal biopsy when the probability of renal impairment due to cast nephropathy is not strongly supported by other data. Thus, in patients with 24-hour urine protein containing mainly light chains and only small amount of albumin, especially if the amount of light chains is > 200 mg/day, and with a serum FLC indicating levels of clonal FLC > 500 mg/L, we do not proceed to a renal biopsy. However, in patients with non-selective proteinuria, those with low levels of FLCs in the serum, those without overt myeloma, in patients with pre-existing renal dysfunction which present with AKI we consider a renal biopsy. A workflow for the investigation of these patients is proposed in Fig. 153.2.

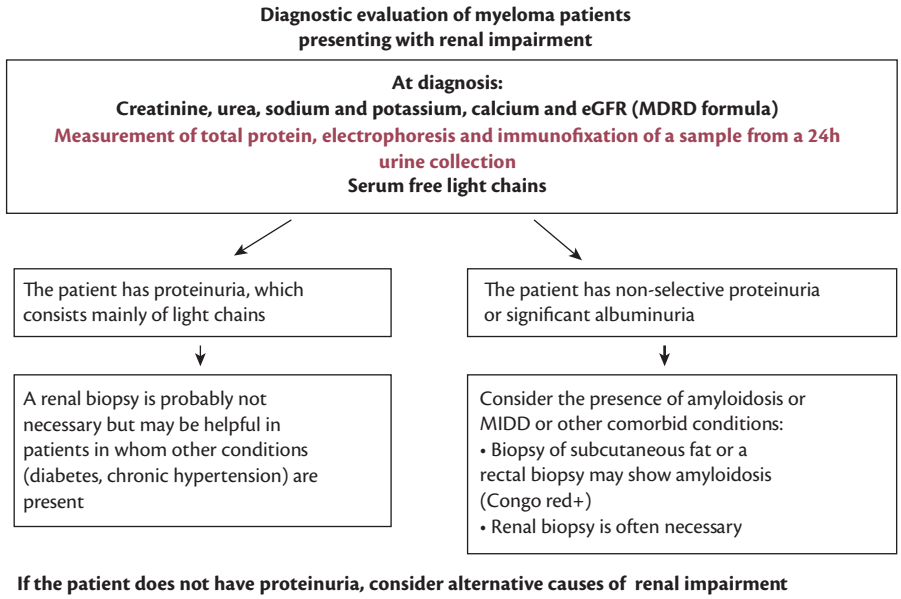

Fig. 153.2 Flowchart of investigation of myeloma/monoclonal gammopathy-related renal impairment.

Evaluation of renal impairment in patients with myeloma

The definition of renal failure in patients with myeloma is not straightforward. A cut-off of serum creatinine of 2 mg/dL is used in the so-called CRAB criteria to define 'symptomatic myeloma' that requires therapy (Durie et al., 2006). This cut-off excludes some patients who may already have significant renal damage due to their monoclonal gammopathy. Thus, it is important to identify early those patients who may already have renal damage from their monoclonal gammopathy, and who may be easier to treat effectively and restore, or salvage, their renal function. Creatinine alone is not a sensitive marker of renal function (Dimopoulos et al., 2010). Calculation of GFR is more accurate. Criteria of acute kidney injury (AKI) such as the RIFLE or the AKIN criteria (Endre, 2008; Endre and Pickering, 2010) may be useful to identify patient who may need immediate intervention, however, these criteria have not been evaluated in patients with myeloma (Dimopoulos et al., 2010c). Other, more precise methods are complicated, have a high cost, and are infrequently used. Cystatin-C measured in the serum is a sensitive marker of renal dysfunction and has been used by nephrologists for some years. In MM patients, Terpos et al. (2007) measured cystatin-C in the serum of newly diagnosed as well in pretreated patients and showed that cystatin-C is elevated in myeloma patients, even in those with normal serum creatinine. Markers of renal damage such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) in serum, and/or urine are increased in patients with monoclonal gammopathies, indicating that renal damage is present very early in the disease course (Dimopoulos et al., 2012a). Thus, suggestions that a decrease of eGFR by ≥ 35% in 1 year, without other identifiable cause, should be considered as an indication for therapy are being considered.

Another significant aspect of management is the evaluation of the response of renal function to treatment of myeloma. For the patient on dialysis, becoming independent of dialysis is a clear indication of improvement. For patients not on dialysis, several criteria have been used, including the normalization of creatinine or the improvement of eGFR by at least 1 or 2 stages of CKD (Dimopoulos et al., 2009a, 2009b, 2010a, 2010b, 2013; Ludwig et al., 2010). The evaluation of myeloma response may also be challenging in patients who do not have measurable disease by serum electrophoresis. Urine quantification of light chains is unreliable in cases of oliguria or when renal function is deteriorating rapidly or during recovery. Serum FLCs have been used for the assessment of patients with light chain amyloidosis as well as patients with oligosecretory disease (Mayo and Johns, 2007); however, in patients with renal impairment and significant load of FLCs the evaluation is not straightforward. In patients with reduced GFR, both kappa and lambda light chains increase and the 'normal' kappa/lambda ratio is different than in patients with normal renal function (Hutchison et al., 2008a, 2008c). The current response criteria for patients with 'measurable' disease do not use the serum FLCs but the urine 24-hour light chain output (Durie et al., 2006). Furthermore, the correlation of urine light chain excretion and of serum FLC levels is not linear, and large variations occur (Abraham et al., 2002).

Management of renal impairment/failure

The management of a patient with myeloma with renal impairment is challenging and requires immediate initiation of therapeutic measures (Dimopoulos et al., 2010c). The basis of the management of the renal impairment is prompt institution of antimyeloma therapy (Table 153.1). Supportive care should be vigorous and additional mechanical means may be justified in selected patients.

Supportive care

Adequate hydration is a key component of supportive care. Although it has been reported that renal failure could be reverted by high fluid intake alone, hydration alone will at best only slightly reduce

Table 153.1 Drugs that are used for the management of patients with multiple myeloma and their use in patients with renal dysfunction

	Clearance by the kidneys	Dose adjustment in RI	Potential of renal toxicity	Data for use in patients on dialysis
Dexamethasone	Yes	No	Low	Yes, safe
Melphalan	Yes	Yes	Low	Yes, with adjustments
Bortezomib	No	No	Low	Yes, safe
Thalidomide	No	No	Low	Yes, safe
Lenalidomide	Yes	Yes	Moderate	Yes, with adjustments
Doxorubicin	No	No	No	Yes
Zoledronic acid	Yes	Yes	Yes	No
Pamidronate	Yes	Yes	Yes	No

the concentration of the pathogenic light chains; hydration should be combined with specific antimyeloma treatment. Additional supportive care measures may include urine alkalinization, although there are no strong supportive clinical data for the use of bicarbonates (MRC Working Party on Leukaemia in Adults, 1984).

The management of hypercalcaemia is crucial and in several cases may be associated with significant improvement of RI. Bisphosphonates are very effective for the management of malignancy-related hypercalcaemia, however, myeloma patients presenting with AKI are at risk for complications such as renal toxicity and subsequent hypocalcaemia when bisphosphonates are administered (Markowitz et al., 2003). Zoledronic acid is contraindicated in patients with a creatinine clearance (CrCl) < 30 mL/min while limited information is available for the use of pamidronate in myeloma patients with a serum creatinine > 3 mg/dL (Dimopoulos et al., 2010c). Thus, close monitoring is needed in these patients, while mild asymptomatic hypercalcaemia should preferably be managed with conservative measures such as hydration. For moderate or severe hypercalcaemia, prompt initiation of antimyeloma therapy, which includes steroids, is indicated. Calcitonin may moderately reduce calcium levels without causing severe hypocalcaemia and without the risk of renal toxicity. When creatinine levels start to improve, then bisphosphonates, in doses adjusted for RI, may be administered (see Table 153.2). The use of furosemide to treat hypercalcaemia is discouraged due to the adverse impact of loop diuretics in the formation of casts in the renal tubule (Sanders and Booker, 1992). Effective supportive care also includes the prompt treatment of infections and the avoidance of agents that contribute to renal damage, such as NSAIDS, aminoglycoside antibiotics, and contrast dyes (Durie et al., 2003).

Mechanical means

An early sustained reduction in serum FLC concentrations is essential in patients with AKI secondary to MM (Hutchison et al., 2011, 2012b); however, the role of a supportive strategy for direct FLC removal in addition to effective chemotherapy has not been clearly defined. Current chemotherapy regimens, especially those based on bortezomib, may induce a rapid and sustained myeloma response; however, despite the rapid reduction of the production of the monoclonal FLCs, high levels of toxic FLCs may still circulate, especially in patients with reduced renal clearance, and thus result in prolonged exposure of the kidneys to the toxic FLCs.

Kappa and lambda FLCs are medium-sized molecules, with molecular weights of 22.5 and 45 kDa respectively. In normal individuals, their serum half-lives are about 3–6 hours. In RI, the serum half-lives of FLCs are prolonged, hence, their serum half-lives may be increased to 2–3 days and their absolute serum concentrations may rise significantly (Hutchison et al., 2008a, 2008c). This prolonged exposure of the kidneys to high FLC levels may further enhance their renal toxicity. Thus, for patients with significantly reduced FLC clearance the direct removal of FLCs from the serum may have a role, always in addition to effective chemotherapy: there is no direct FLC removal technique that can substitute effective chemotherapy. Methods that have been used for FLC removal include plasma exchange and the use of specific dialysis filters.

Plasma exchange

In theory, rapid removal of nephrotoxic light chains with plasma exchange, in combination with antimyeloma therapy, could prevent further renal damage and improve renal function. Two studies suggested that plasma exchange was beneficial (Pozzi et al., 1987; Zucchelli et al., 1988); however, a small prospective comparison of forced diuresis and chemotherapy (10 patients) versus forced diuresis, chemotherapy, and plasma exchange (11 patients) found only a trend in favour of the plasma exchange (Johnson et al., 1990). A larger randomized trial failed to show clear evidence that plasma exchange improved the outcome in patients with MM and AKI (Clark et al., 2005); this study, however, has been criticized for the lack of FLC measurements (which were not available) and the lack of histologic evidence of cast nephropathy (since renal biopsy was available in very few patients). Unfortunately, a randomized controlled trial of plasma exchange in AKI secondary to MM in the United Kingdom failed to recruit the target population. Thus, the possibility that a subgroup of patients might benefit from plasma exchange cannot be ruled out. Bladé and Rosinol (2005) supported that patients with renal failure severe enough to require dialysis do not benefit from plasma exchange. This is in agreement with older findings which associated the severity of myeloma cast formation with the irreversibility of renal failure, even in patients undergoing plasma exchange (Pozzi et al., 1987; Johnson et al., 1990). However, in a recent report from the Mayo Clinic, plasma exchange in combination with bortezomib-based chemotherapy, in a limited number of patients, was associated with high rates of renal recovery (Burnette et al., 2011).

Table 153.2 Dose modifications for drugs that are used for the management of patients with multiple myeloma in patients with renal dysfunction

	CrCl > 60 mL/min	CrCl 30–59 mL/min	CrCl 15–29 mL/min	CrCl < 15 mL/min	Data for use in patients on dialysis
Dexamethasone	Various	No dose modification needed	No dose modification needed	No dose modification needed	No dose modification needed
Melphalan	Oral melphalan: 0.15 to 0.25 mg/kg/day for 4–7 days High-dose melphalan: 200 mg/m^2	Oral melphalan: reduction by 25% (0.11 to 0.19 mg/kg/day for 4–7 days High-dose melphalan: 140 mg/m^2	Oral melphalan: reduction by 25% (0.11 to 0.19 mg/kg/day for 4–7 days High-dose melphalan: 140 mg/m^2	Oral melphalan: reduction by 50% (0.0175 to 0.125 mg/kg/day for 4 to 7 days). High-dose melphalan: 140 mg/m^2	Oral melphalan: reduction by 50% (0.0175 to 0.125 mg/kg/day for 4 to 7 days). High-dose melphalan: 140 mg/m^2
Bortezomib	1.3 mg/m^2 days 1, 4, 8, 11 or weekly regimens	No dose modification needed	No dose modification needed	No dose modification needed	No dose modification needed
Thalidomide	50–200 mg/day	No dose modification needed	No dose modification needed	No dose modification needed	No dose modification needed
Lenalidomide	25 mg/day	10 mg daily, can be increased to 15 mg/day if no toxicity occurs	15 mg once every other day, than can be adjusted to 10 mg per day	5 mg/day	5 mg/day
Carfilzomib	20 mg/m^2 cycle 1; 27 mg/m^2 cycle 2 and on	No dose modification needed	No dose modification needed	No dose modification needed	No dose modification needed
Doxorubicin	Various	No dose modification needed	No dose modification needed	No dose modification needed	No dose modification needed
Cyclophosphamide	Various	No dose modification needed	No dose modification needed	No dose modification needed	No dose modification needed
Zoledronic acid	4 mg	CrCl 50–60 mL/min: 3.5 mg CrCl 40–49 ml/min: 3.3 mg CrCl 30–39 ml/min: 3 mg	Not recommended	Not recommended	No
Pamidronate	30–90 mg	Patients with renal impairment (serum creatinine > 3.0 mg/dL) have not been studied	Limited pharmacokinetic data exist in patients with creatinine clearance <30 mL/min	Limited pharmacokinetic data exist in patients with creatinine clearance <30 mL/min	Limited pharmacokinetic data exist in patients with creatinine clearance < 30 mL/min

The reason for the relatively disappointing results of plasma exchange may be the physical properties of the light chains. As a consequence of their molecular weight, FLCs re-equilibrate relatively freely between intra- and extravascular compartments and thus, approximately 80% of FLCs are extravascular. Therefore, despite the effective clearance of FLCs from the serum with plasma exchange, the short duration of each session limits the clearance to the extravascular compartment. An increased 'dose' of plasma exchange may thus be required in order to provide a significant clinical benefit by removing FLCs also from the extravascular compartment (Cserti et al., 2007; Hutchison et al., 2007a). We believe that in some patients with non-oliguric AKI, early initiation of a plasma exchange programme and effective chemotherapy may be of some benefit, but these patients should be carefully selected (Dimopoulos et al., 2010c; Burnette et al., 2011).

Free light-chain removal with dialysis filters

The removal of FLCs with dialysis is another alternative approach and a new haemodialysis membrane which removes the circulating light chains more efficiently has been recently developed. This membrane allows for the removal of FLCs through its larger pores which allow the removal of molecules up to 60–65 kD. However, after effective removal of a solute from the serum, the clearance of the extravascular compartment is dependent on the duration of the removal; thus, the use of this strategy for the removal of FLCs also requires prolonged treatment (Ward et al., 2006). A small study investigating haemodialysis with a protein-leaking dialyser indicated that large reductions in the concentration of serum FLCs could be obtained (Hutchison et al., 2007a). The same group also investigated the use of high cut-off haemodialysis in combination with systemic treatments and found that 13 of 18 patients with renal failure became dialysis independent (Hutchison et al., 2007b). Renal recovery was also associated with improved survival ($P < 0.02$). Although promising, these results need further confirmation in larger studies. Also, it should be noted that in some patients the FLCs can form large polymers and cannot be removed through the pores of the membrane. Currently a European multicentre randomized (EuLITE, NCT00700531) controlled study is

evaluating the hypothesis that FLC removal by high cut-off haemodialysis may increase renal recovery rates from myeloma kidney beyond effective chemotherapy alone.

Renal replacement with dialysis

Despite the improvements in recent years, the mortality rate among patients with MM and dialysis-dependent renal failure during the first 2 months from diagnosis is about 30% (Torra et al., 1995). The response rate to chemotherapy in patients with MM on long-term dialysis programme ranges from 40% to 60% (Misiani et al., 1987; Iggo et al., 1989; Korzets et al., 1990; Torra et al., 1995); thus, the presence of renal failure may not have a negative impact on the response to chemotherapy per se. On the other hand, if patients who die within the first 2 months from diagnosis are excluded, the median survival of patients with MM and non-reversible end-stage renal failure is almost 2 years and 30% of them survive for > 3 years (Johnson et al., 1980; Torra et al., 1995). This suggests that even patients with MM, in need for long-term dialysis may have a long survival. However, another important aspect is the quality of life of this population of patients. In two series, the average of hospitalization days was 12 and 19 days per patient-year, respectively (Johnson et al., 1980; Torra et al., 1995). In one of these studies, patients who survived for > 1 year spend < 10 days per year in hospital, a figure similar to that observed in patients on chronic haemodialysis because of diabetic nephropathy (Torra et al., 1995). It seems that there are no long-term differences between chronic peritoneal dialysis (CAPD) and haemodialysis, although patients on CAPD are at a higher risk of developing bacterial peritonitis (Shetty and Oreopoulos, 1995). In summary, long-term dialysis is a worthwhile treatment for patients with MM and end-stage renal failure.

Specific antimyeloma therapy

Antimyeloma therapy is the mainstay of treatment for patients presenting with myeloma-related renal impairment. Immediate institution of antimyeloma therapy is necessary in order to reduce the load of the toxic light chains to the kidney and improve renal function.

High-dose dexamethasone-based regimens (such as VAD or similar) have been traditionally considered based on their rapid antimyeloma effect (Alexanian et al., 1990; Kastritis et al., 2007). However, after the introduction of novel agents in the late 1990s, the therapeutics of myeloma have substantially changed. These novel agents (thalidomide, lenalidomide, and bortezomib) used in combination with dexamethasone (with or without other drugs) are associated with high response rates and a fast myeloma response. Even with these of novel agents, steroids may have a significant role. They may rapidly reduce calcium levels, and thus are crucial for the management of hypercalcaemia. Furthermore, in a recent retrospective analysis in newly diagnosed myeloma patients who presented with and who received novel agents (thalidomide, bortezomib, or lenalidomide), higher doses of steroids (dexamethasone) were independently associated with shorter time to renal recovery, although were not independently associated with higher probability of renal response (Dimopoulos et al., 2013).

Thalidomide, an immunomodulatory drug (IMiD), has been used in patients with renal impairment for several years, although there are not many reports on either antimyeloma efficacy or on renal improvement (Tosi et al., 2004, 2010; Kastritis et al., 2007; Dimopoulos et al., 2013). This drug is not excreted by the kidneys and can be used even in patients undergoing dialysis without dose modifications (Eriksson et al., 2003). However, some toxicities, such as unexplained hyperkalaemia, may be of concern, especially in patients undergoing dialysis (Harris et al., 2003; Fakhouri et al., 2004). When used with high-dose dexamethasone, thalidomide may be associated with a significant improvement of the renal function in a high proportion of myeloma patients presenting with renal insufficiency (Tosi et al., 2004, 2010; Kastritis et al., 2007; Dimopoulos et al., 2013) (Table 153.3).

Lenalidomide is a second-generation IMiD that has very similar structure but very different pharmacokinetic and toxicity profile than thalidomide (Anderson and Prince, 2005). Lenalidomide is excreted by the kidneys and thus, the dose should be adjusted according to GFR (Chen et al., 2007). In the initial phase 3 trials comparing lenalidomide/dexamethasone (Len/Dex) with dexamethasone/placebo in patients with relapsed or refractory MM, patients with a serum creatinine > 2.5 mg/dL were excluded, but several patients with a CrCl < 60 mL/min and some with a CrCl < 30 mL/min were treated with Len/Dex. Dimopoulos et al. (2010a) analysed 353 patients who received Len/Dex, including patients with moderate (CrCl < 60 mL/min, 82 patients) or severe (< 30 mL/min, 16 patients) renal impairment. There were no significant differences in response rates or quality of response among patients with different degrees of renal dysfunction. Time to progression (TTP) and progression-free survival (PFS) were not significantly different, although there was a trend towards a shorter survival in patients with moderate or severe renal impairment. Importantly, improvement of renal dysfunction by at least one level (i.e. mild or no impairment from moderate RI, or severe to moderate or better) was observed in 72% of patients with moderate-to-severe renal impairment who received Len/Dex. However, thrombocytopenia was significantly more common and dose reductions were required more often in patients with moderate or severe impairment who received Len/Dex. Similarly, in an expanded access programme of lenalidomide given in patients with relapsed or refractory MM, haematologic toxicity (mainly thrombocytopenia) and dose reduction were more common on patients with moderate RI (Reece et al., 2006). In several small retrospective series, patients with reduced GFR have been treated with lenalidomide-based regimens and it seems that when lenalidomide dose was adjusted according to CrCl the toxicity was not excessive (Chen et al., 2007; de la Rubia et al., 2010; Dimopoulos et al., 2010b; Klein et al., 2011) while some patients with moderate or severe reductions had an improvement in their renal dysfunction (Dimopoulos et al., 2010b; Klein et al., 2011). In a small series of patients undergoing dialysis, lenalidomide in adjusted doses induced significant response rates and one of 15 patients became dialysis independent (de la Rubia et al., 2010). In a retrospective analysis, comparing different novel agent-based first-line regimens in MM patients with renal impairment, Len/Dex was associated with a clinically significant GFR improvement in 43% of patients, most of whom were of advanced age (Dimopoulos et al., 2013). Thus, lenalidomide-based therapy can be considered for the treatment of patients with MM, even of those with moderate or severe renal impairment, provided that the dose is appropriately adjusted.

Bortezomib is the first in class proteasome inhibitor that has been approved for the treatment of patients with MM. This drug has significant antimyeloma activity and also several advantages especially for patients with RI. Bortezomib has a half-life independent

Table 153.3 Activity of novel agent-based regimens in patients with MM presenting with renal impairment

	Number of patients with RI	Patients under dialysis	Disease status	Myeloma response	Response of RI	Comments
Thalidomide studies						
Fakhouri et al., 2004	7	1	Rel/ref	3/7 CR, 1 PR, 3 MR		One patient presented with severe hyperkalaemia (> 8 mmol/L) on two occasions during therapy with 400 mg/d thalidomide.
Tosi et al., 2004	20 with sCr > 1.5 mg/dL and CrCl < 60 mL/min	3	Rel/ref	PR 45%	60% achieved sCr < 1.5 mg/dL	Toxicity manageable and predictable
Kastritis et al., 2007	13 with sCr > 2 mg/dL	4	Newly diagnosed	65%	77% sCr <1.5 mg/dL 3/4 dialysis patients → dialysis independent	Toxicity manageable and predictable
Tosi et al., 2010	31 with CrCl < 50 mL/min	7	Newly diagnosed	74% (10% CR)	55% CrCl > 50 mL/min 2 of 7 → dialysis independent	10% developed DVT—one patient developed extensive skin rash
Dimopoulos et al., 2013	62	4	Newly diagnosed	ORR: 63%	Major renal response (≥ renal PR): 55% Renal CR: 53% 2/4 patients became dialysis independent	Toxicity manageable and predictable Median time to renal response 2.7 months (82 days)
Lenalidomide studies						
Chen et al., 2007	17	6	Non-malignant conditions	NR	NR	Pharmacokinetics of lenalidomide substantially affected by moderate and severe RI Dose adjustments necessary
Reece et al., 2006	23	0	Rel/ref	Response rate, PFS, OS similar in patients with normal vs ↑ creatinine level	NR	Expanded access study in MM Patients with ↑creatinine level had lower baseline PLT counts, and required more PLT transfusions
Niesvizky et al., 2007	14 with CrCl < 40 mL/min	0	Newly diagnosed	NR	3/14 had an increase of CrCl to > 70 mL/min	Renal dysfunction resulted in lenalidomide dose reduction Baseline CrCl < 40 mL/min was associated with grade ≥3 myelosuppression and the need to reduce lenalidomide dosage
Dimopoulos et al., 2010a	98 (82 patients with CrCl < 60 mL/min, and 16 with < 30 mL/min)	0	Rel/ref	ORR & quality of response similar to patients without RI	Improvement of renal dysfunction by at least one level in 72%	Trend for shorter survival in patients with moderate or severe RI. Thrombocytopenia was significantly more common and dose reductions were required more often in patients with moderate or severe
de la Rubia et al., 2010	15	15	Rel/ref	4 CR, 1 VGPR, 4 PR,	1 pt became dialysis independent	Len adjusted to RI (15 mg 3 × week or 5 mg QD) 4 pts died due to infectious complications

(*Continued*)

Table 153.3 Continued

	Number of patients with RI	Patients under dialysis	Disease status	Myeloma response	Response of RI	Comments
Dimopoulos et al., 2010b	12	1	Rel/ref	61%	In 40% of patients with RI improved	Lenalidomide dose was adjusted according to renal function—no excessive toxicity.
Klein et al., 2010	33	5	Rel/ref	3% CR, 12% VGPR, 49% ORR	27% showed an improvement of RI	OS was similar for patients with or without RI
Dimopoulos et al., 2013	28	0	Newly diagnosed	ORR 82%	Major renal response (≥ renal PR): 43% Renal CR: 36% Increase of median eGFR from 49 to 85 mL/min/1.73m^2	Len adjusted to RI Manageable and no excess toxicity
Bortezomib studies						
Jagannath et al., 2005	52 with CrCl < 50 mL/min	0	Rel/ref	25%	NR	Bortezomib effective Manageable toxicities
San Miguel et al., 2008	58 with CrCl	0	Rel/ref	47%	NR	Trend for shorter OS in patients with moderate/sever RI compared to no/mild RI
Mulkerin et al., 2007	43	9	Advanced cancer	NR	NR	Bortezomib clearance independent of renal function The overall AE profile in dialysis pts was similar to that in controls and in pts with mild-to-severe impairment, although renal and metabolic AEs appeared more common in dialysis pts
Ailawadhi et al., 2007	28 with eGFR <60 mL/min	3	Rel/ref and newly diagnosed	ORR 57%	NR	No significant association between renal function and response to treatment
Dimopoulos et al., 2009b	46	9	Rel/ref and newly diagnosed	76%	Reversal of renal failure in 59% 2/9 became dialysis independent	previously untreated disease was associated with renal response Light chain myeloma was associated with a shorter time to renal response 4 out of 9 patients who were rated as having stable disease achieved a renal response as well as 1 of 8 patients who had progressive disease
Ludwig et al., 2010	68	9	Rel/ref and newly diagnosed	ORR 72% (38% CR/nCR, 15% VGPR, 13% PR)	62% had a renal response Median GFR increased from 20.5 mL/min to 48.4 mL/min. 3/9 became dialysis independent	Significant improvement in renal function (renal CR) correlated with baseline GFR and myeloma response
Morabito et al., 2010	117 (10% had CrCl 50–80 mL/min)	14	Rel/ref and newly diagnosed	ORR 73% (19% CR, 8% nCR 17% very good VGPR	> 80 mL/min in 41% 3/14 discontinued dialysis	RI improvement more frequently in previously untreated patients and in those with mild to moderate RI ORR was similar across renal subgroups (severe vs moderate vs mild RI)

(*Continued*)

Table 153.3 Continued

	Number of patients with RI	Patients under dialysis	Disease status	Myeloma response	Response of RI	Comments
Bladé et al., 2008	193 patients with renal insufficiency (CrCl < 60 mL/min—no patient with CrCl < 30 mL/min)	0	Rel/ref	49% in B+PLD 39% in B alone	Statistically significant improvement in renal function (increase in CrCl)	Median TTP in patients with renal insufficiency Bortezomib + PLD 10.9 months Bortezomib alone 6.5 months Grade 3/4 anaemia, diarrhoea and pneumonia more common in patients with RI
Dimopoulos et al., 2009c	111 (GFR < 50 mL/min)	0	Newly diagnosed	68% (31% CR)	44% of patients improved to GFR > 60 mL/min	Retrospective analysis of phase 3 trial Rates of grade ≥ 4 AEs appeared somewhat higher in patients with GFR > 50 mL/min. Patients achieving renal impairment reversal had a better safety profile than those with irreversible RI 37% of patients with GFR < 50 mL/min who did not respond to VMP had RI reversal.
Dimopoulos et al., 2013	43 (eGFR < 60 mL/min)	6 (14%)	Newly diagnosed	ORR: 81%	Major renal response (≥ renal PR): 77% Renal CR: 67% 3/6 patients became independent of dialysis	Median time to renal response 41 days

RI = renal impairment; CR = complete response; ORR = overall response rates; MR: minor response; PR = partial response; Rel/ref: relapsed/refractory.

of renal clearance (Mulkerin et al., 2007) and data from the initial phase 2 and 3 trials as well as data from patients undergoing dialysis indicated that bortezomib is safe to use in patients with renal dysfunction (Jagannath et al., 2005; San-Miguel et al., 2008), even those undergoing dialysis (Chanan-Khan et al., 2007), without need for dose adjustments. In addition, bortezomib may reduce the inflammation in myeloma kidney disease and inhibit production of proinflammatory factors (Ying et al., 2011). There are data which indicate that the favourable activity of bortezomib in the context of RI may also be due to a 'protective' effect on renal cells and inhibition of inflammatory and fibrotic cascades within kidney microenvironment. Thus, *in vitro*, bortezomib induced survival signals in a proximal tubular renal cell line (Sarkozi et al., 2008). Proteasome inhibition with bortezomib partially blocked light-chain-induced MCP-1 by Src kinase-dependent activation of the NF-κB pathway, and this may be associated with an inhibition of proinflammatory and profibrotic activity within renal microenvironment (Ying et al., 2011). However, the most important advantages of bortezomib are its rapid and significant antimyeloma action and its non-renal metabolism (Pekol et al., 2005; Labutti et al., 2006).

Reversal of renal dysfunction and significant improvement of renal function has been observed in several studies and even some patients on dialysis can became dialysis independent after treatment with bortezomib (Chanan-Khan et al., 2007). This effect has been observed when bortezomib is used either in combination with dexamethasone or with other agents such as doxorubicin, cyclophosphamide, or thalidomide. It has been consistently reported that about 20–30% of patients on dialysis may become dialysis independent (Ludwig et al., 2007; Roussou et al., 2008; Morabito et al., 2010). Bladé et al. (2008) analysed a phase 3 study comparing bortezomib alone to bortezomib plus liposomal doxorubicin in patients with relapsed or refractory MM in which 193 patients with RI (CrCl < 60 mL/min but none had a CrCl < 30 mL/min) were included. A steady improvement in CrCl from a median of about 35–60 mL/min after therapy was observed, while other outcome measures such as median TTP and PFS were not significantly different among patients with or without renal impairment across treatment groups. Data from the phase 3 study VISTA, comparing VMP to MP in patients ineligible for ASCT, indicated that the rate of renal recovery was higher with VMP (44% of patients with baseline GFR < 50 mL/min improved to > 60 mL/min), compared to MP (34%). This difference was more pronounced in patients with more severe impairment (GFR < 30 mL/min). Time to renal improvement in patients with baseline GFR < 50 mL/min was also significantly shorter with VMP than MP (2.1 months vs 2.4 months) (Dimopoulos et al., 2009a). In a prospective phase 2 study including previously treated and untreated patients with MM-related acute RI, 68 patients received bortezomib with doxorubicin and dexamethasone (Ludwig et al., 2010). Tumour response was recorded in 84% (≥ VGPR in 62%). Median GFR increased from 20 mL/min to 48.8 mL/min, complete renal response (defined as GFR ≥ 60 mL/min) was observed in 36%, and the overall renal response was 72%. In addition three of nine dialysis-dependent patients became independent of the procedure.

Renal responses in patients treated with bortezomib-based regimens tend to occur rapidly, generally within the initial two

to three cycles of treatment and this is consistent across several studies (Dimopoulos et al., 2009a, 2009b; Ludwig et al., 2010). Another common observation is that renal response correlates with tumour response and is more common in newly diagnosed patients (Dimopoulos et al., 2009b; Ludwig et al., 2010). However, it is also interesting that some patients who do not achieve an objective myeloma response may also have a significant improvement in their renal function (Dimopoulos et al., 2009a, 2009b; Ludwig et al., 2010). In retrospective studies, bortezomib-based therapy in newly diagnosed patients with reduced GFR was superior in terms of renal improvement and in time to the achievement of this improvement when compared to high-dose, dexamethasone-based, IMiDs-based therapy (thalidomide or lenalidomide) (Roussou et al., 2010), or separately to thalidomide or lenalidomide (Dimopoulos et al., 2013). Overall, significant improvement of renal function was observed in 77–82% of patients treated with bortezomib-based therapy. Furthermore, this improvement seems to be independent of the dose of steroids, although higher dose of dexamethasone may be associated with shorter time to renal recovery (Dimopoulos et al., 2013).

The International Myeloma Working Group has published a consensus statement regarding the management of patients with MM presenting with renal impairment (Dimopoulos et al., 2010c) and recommends the use of bortezomib-based regimens as first choice. The combination of bortezomib with thalidomide and dexamethasone is considered, although the data on this regimen are limited. Lenalidomide is also considered, although data on this regimen come mainly from patients with mild-to-moderate renal impairment, at doses adjusted to renal function. Considering the lack of randomized trials comparing different regimens but based on the available data we also recommend bortezomib/dexamethasone-based regimens for MM patients presenting with renal impairment as first choice. Lenalidomide/dexamethasone-based regimens can also be considered given the high response rates.

Stem cell transplantation in patients with renal impairment

High-dose therapy with autologous stem cell transplantation is a major treatment option in patients with myeloma who are usually < 65 years of age. This treatment modality is not given immediately after diagnosis of MM, but after an induction phase, which nowadays, is usually based on novel agents. Thus, several patients who initially presented with severe renal impairment may have a significant improvement of their renal function by the time of transplant. However, high-dose therapy (HDT) and autologous stem cell transplantation (ASCT) may still be recommended in eligible patients.

Patients with persisting renal impairment were excluded from most randomized trials, but HDT and ASCT is feasible in these patients, including those who require dialysis (San Miguel et al., 2000; Badros et al., 2001). Current data indicate that the quality of stem cell collection and engraftment are unaffected (Badros et al., 2001; Lee et al., 2004). A reduced dose of melphalan (140 mg/m^2) is used in patients with low GFR or undergoing dialysis; the reduced dose does appear as effective as 200 mg/m^2, probably with less toxicity (Badros et al., 2001) but it has not been tested in a randomized study. Furthermore, melphalan kinetics are very difficult to predict, even in patients with normal renal function. However, HDT in patients with reduced GFR is associated with an increased risk of toxicity, which seems to increase with the degree of renal dysfunction (Tricot et al., 1996; Carlson et al., 2005). In patients with persisting renal impairment, rates of transplant-related mortality between 4% and 29% were reported (San Miguel et al., 2000; Badros et al., 2001; Lee et al., 2004; Knudsen et al., 2005); in comparison, transplant-related mortality is < 1% in patients who recovered a normal renal function after initial therapy (Knudsen et al., 2005).

The impact of HDT/ASCT on renal function has not been well studied. Retrospective studies indicate that improvement of CrCl of at least 25% above baseline is about 30% (Hutchison et al., 2007b) after HDT; dialysis independence might also occur in about 15–25% of HDT-treated patients who were on dialysis at the time of HDT (Sirohi et al., 2001; Parikh et al., 2009). In the largest series of dialysis-dependent patients, 5-year event-free and overall survivals were 24% and 36% (Lee et al., 2004), but the absence of prospective studies prevents any definitive conclusion. Given the availability of novel agents which have improved outcomes, including the rates of renal improvement, current data on ACST should be evaluated carefully. It is our practice to offer HDT with ASCT to patients who are eligible for the procedure, even those under dialysis, but at the dose of 140 mg/m^2 for melphalan (Dimopoulos et al., 2010c).

Prognosis of patients with multiple myeloma presenting with renal impairment

Renal impairment reflects advanced disease and high tumour burden in patients with myeloma cast nephropathy (Dimopoulos et al., 2010c; Hutchison et al., 2012a); in those with LCDD or AL amyloidosis, however, there is only a small or modest in size plasma cell clone in the bone marrow (Merlini and Stone, 2006). Patients with myeloma and reduced GFR are mostly rated as stage III by the International Scoring System (ISS), due to the elevated levels of beta-2-microglobulin which are partially a result of reduced excretion. Nevertheless, this is associated with significantly shorter survival (Dimopoulos et al., 2012c). Renal recovery may be associated with improved survival; however, this has not been confirmed in all series (Dimopoulos et al., 1990; Bladé et al., 1998; Knudsen et al., 2000; Kastritis et al., 2007).

Patients who present with AKI have increased early mortality, reaching up to 30% within the first 2 months in some series (Bladé et al., 1998; Augustson et al., 2005). Indeed, the risk of early death increases more than fourfold for patients with more severe renal impairment (Dimopoulos et al., 2012b). Furthermore, renal damage adversely affects the outcome of myeloma patients in various ways: drug dosing is often complicated while many potentially nephrotoxic but useful drugs are deferred; patients with renal failure, of any cause, are also more susceptible to infections and electrolyte imbalance complications, are more likely to require prolonged hospitalization and their quality of life may be severely compromised. However, the prognosis has improved significantly over the past decade, mainly due to the availability of more effective treatments for MM and improvements in supportive care. A major improvement was observed after the year 2000, when thalidomide, the first of the novel agents, became widely available, and also after 2005 when bortezomib as first-line therapy was used in several patients (Dimopoulos et al., 2012b)—this improvement is more pronounced in patients with more severe impairment (eGFR < 30 mL/min) indicating the importance of the use of novel agents with increased antimyeloma activity.

Renal transplantation in patients with myeloma-related end-stage renal disease

Renal transplantation in patients with myeloma is rare due to the nature of the disease: patients with MM die due to their underlying malignancy in a median of 3–5 years after the diagnosis. Furthermore, patients with MM are at high risk for infections, may experience several relapses in which the allograft is exposed to toxic light chains, and the use of immunosuppression may be problematic (De Lima et al., 1981; Walker and Bear, 1983; van Bommel, 1996). However, there is an increasing number of patients with MM who attain long-lasting responses, including several patients who may have a complete response of their underlying myeloma for several years. Thus, a renal transplantation could be considered for some patients (Dagher et al., 1996; Tsakiris et al., 2010; Bansal et al., 2012). A combination of ASCT with renal transplantation has also been reported (Spitzer et al., 1999, 2011). Still, there are only limited data about renal transplantation in patients with myeloma and current information needs to be reviewed carefully, especially in light of the recent improvements in the outcome of patients with MM (Kumar et al., 2008; Kastritis et al., 2009).

References

Abraham, R. S., Clark, R. J., Bryant, S. C., *et al.* (2002). Correlation of serum immunoglobulin free light chain quantification with urinary Bence Jones protein in light chain myeloma. *Clin Chem*, 48(4), 655–7.

Ailawadhi, S., Mashtare, T. L., Coignet, M. V., *et al.* (2007). Renal dysfunction does not affect clinical response in multiple myeloma (MM) patients treated with bortezomib-based regimens. *ASH Annual Meeting Abstracts*, 110(11), 1477.

Alexanian, R., Barlogie, B., and Dixon, D. (1990). Renal failure in multiple myeloma. Pathogenesis and prognostic implications. *Arch Intern Med*, 150(8), 1693–5.

Anderson, K. C. and Prince, H. M. (2005). Lenalidomide and thalidomide: an evolving paradigm for the management of multiple myeloma. *Semin Hematol*, 42(4 Suppl 4), S1–2.

Anonymous (1959). Multiple myeloma with renal failure. *Am J Med*, 26(3), 485–95.

Augustson, B. M., Begum, G., Dunn, J. A., *et al.* (2005). Early mortality after diagnosis of multiple myeloma: analysis of patients entered onto the United Kingdom Medical Research Council trials between 1980 and 2002—Medical Research Council Adult Leukaemia Working Party. *J Clin Oncol*, 23(36), 9219–26.

Badros, A., Barlogie, B., Siegel, E., *et al.* (2001). Results of autologous stem cell transplant in multiple myeloma patients with renal failure. *Br J Haematol*, 114(4), 822–9.

Bansal, T., Garg, A., Snowden, J. A., *et al.* (2012). Defining the role of renal transplantation in the modern management of multiple myeloma and other plasma cell dyscrasias. *Nephron Clin Pract*, 120(4), c228–35.

Bartels, E. D., Brun, G. C., Gammeltoft, A., *et al.* (1954). Acute anuria following intravenous pyelography in a patient with myelomatosis. *Acta Med Scand*, 150(4), 297–302.

Batuman, V. (2007). Proximal tubular injury in myeloma. *Contrib Nephrol*, 153, 87–104.

Batuman, V., Dreisbach, A. W., and Cyran, J. (1990). Light-chain binding sites on renal brush-border membranes. *Am J Physiol*, 258(5 Pt 2), F1259–65.

Batuman, V. and Guan, S. (1997). Receptor-mediated endocytosis of immunoglobulin light chains by renal proximal tubule cells. *Am J Physiol*, 272(4 Pt 2), F521–30.

Batuman, V., Verroust, P. J., Navar, G. L., *et al.* (1998). Myeloma light chains are ligands for cubilin (gp280). *Am J Physiol*, 275(2 Pt 2), F246–54.

Blackman, S. S., Barker, W. H., Buell, M. V., *et al.* (1944). On the pathogenesis of renal failure associated with multiple myeloma. Electrophoretic and chemical analysis of protein in urine and blood serum. *J Clin Invest*, 23(2), 163–6.

Bladé, J., Fernandez-Llama, P., Bosch, F., *et al.* (1998). Renal failure in multiple myeloma: presenting features and predictors of outcome in 94 patients from a single institution. *Arch Intern Med*, 158(17), 1889–93.

Bladé, J. and Rosinol, L. (2005). Renal, hematologic and infectious complications in multiple myeloma. *Best Pract Res Clin Haematol*, 18(4), 635–52.

Bladé, J., Sonneveld, P., San Miguel, J. F., *et al.* (2008). Pegylated liposomal doxorubicin plus bortezomib in relapsed or refractory multiple myeloma: efficacy and safety in patients with renal function impairment. *Clin Lymphoma Myeloma*, 8(6), 352–5.

Brodwall, E. K., Knutsen, S. B., and Myhre, J. R. (1956). Acute renal failure following intravenous pyelography in cases of myelomatosis. *Acta Med Scand*, 156(4), 263–6.

Burnette, B. L., Leung, N., and Rajkumar, S. V. (2011). Renal improvement in myeloma with bortezomib plus plasma exchange. *N Engl J Med*, 364(24), 2365–6.

Buxbaum, J. and Gallo, G. (1999). Nonamyloidotic monoclonal immunoglobulin deposition disease. Light-chain, heavy-chain, and light- and heavy-chain deposition diseases. *Hematol Oncol Clin North Am*, 13(6), 1235–48.

Carlson, K. (2005). Melphalan 200 mg/m2 with blood stem cell support as first-line myeloma therapy: impact of glomerular filtration rate on engraftment, transplantation-related toxicity and survival. *Bone Marrow Transplant*, 35(10), 985–90.

Chanan-Khan, A. A., Kaufman, J. L., Mehta, J., *et al.* (2007). Activity and safety of bortezomib in multiple myeloma patients with advanced renal failure: a multicenter retrospective study. *Blood*, 109(6), 2604–6.

Chen, N., Lau, H., Kong, L., *et al.* (2007). Pharmacokinetics of lenalidomide in subjects with various degrees of renal impairment and in subjects on hemodialysis. *J Clin Pharmacol*, 47(12), 1466–75.

Clark, W. F., Stewart, A. K., Rock, G. A., *et al.* (2005). Plasma exchange when myeloma presents as acute renal failure: a randomized, controlled trial. *Ann Intern Med*, 143(11), 777–84.

Comenzo, R. L., Zhang, Y., Martinez, C., *et al.* (2001). The tropism of organ involvement in primary systemic amyloidosis: contributions of Ig V(L) germ line gene use and clonal plasma cell burden. *Blood*, 98(3), 714–20.

Cserti, C., Haspel, R., Stowell, C., *et al.* (2007). Light-chain removal by plasmapheresis in myeloma-associated renal failure. *Transfusion*, 47(3), 511–14.

Dagher, F., Sammett, D., Abbi, R., *et al.* (1996). Renal transplantation in multiple myeloma. Case report and review of the literature. *Transplantation*, 62(11), 1577–80.

De la Rubia, J., Roig, M., Ibanez, A., *et al.* (2010). Activity and safety of lenalidomide and dexamethasone in patients with multiple myeloma requiring dialysis: a Spanish multicenter retrospective study. *Eur J Haematol*, 85(4), 363–5.

De Lima, J. J., Kourilsky, O., Meyrier, A., *et al.* (1981). Kidney transplant in multiple myeloma. Early recurrence in the graft with sustained normal renal function. *Transplantation*, 31(3), 223–4.

DeFronzo, R. A., Cooke, C. R., Wright, J. R., *et al.* (1978). Renal function in patients with multiple myeloma. *Medicine (Baltimore)*, 57(2), 151–66.

Dimopoulos, M., Alegre, A., Stadtmauer, E. A., *et al.* (2010a). The efficacy and safety of lenalidomide plus dexamethasone in relapsed and/or refractory multiple myeloma patients with impaired renal function. *Cancer*, 116(16), 3807–14.

Dimopoulos, M. A., Christoulas, D., Kastritis, E., *et al.* (2012a). Tubular damage is ubiquitous in newly-diagnosed patients with multiple myeloma: comparison of three urinary and two serum markers of kidney injury. *ASH Annual Meeting Abstracts*, 120(21), 2919.

Dimopoulos, M. A., Christoulas, D., Roussou, M., *et al.* (2010b). Lenalidomide and dexamethasone for the treatment of refractory/

relapsed multiple myeloma: dosing of lenalidomide according to renal function and effect on renal impairment. *Eur J Haematol*, 85(1), 1–5.
Dimopoulos, M. A., Delimpasi, S., Katodritou, E., *et al.* (2012b). Significant improvement of the survival of patients with multiple myeloma presenting with severe renal impairment after the introduction of novel agents. *ASH Annual Meeting Abstracts*, 120(21), 948.
Dimopoulos, M. A., Kastritis, E., Michalis, E., *et al.* (2012c). The International Scoring System (ISS) for multiple myeloma remains a robust prognostic tool independently of patients' renal function. *Ann Oncol*, 23(3), 722–9.
Dimopoulos, M. A., Richardson, P. G., Schlag, R., *et al.* (2009a). VMP (bortezomib, melphalan, and prednisone) is active and well tolerated in newly diagnosed patients with multiple myeloma with moderately impaired renal function, and results in reversal of renal impairment: cohort Analysis of the Phase III VISTA Study. *J Clin Oncol*, 27(36), 6086–93.
Dimopoulos, M. A., Roussou, M., Gavriatopoulou, M., *et al.* (2009b). Reversibility of renal impairment in patients with multiple myeloma treated with bortezomib-based regimens: identification of predictive factors. *Clin Lymphoma Myeloma*, 9(4), 302–6.
Dimopoulos, M. A., Roussou, M., Gkotzamanidou, M., *et al.* (2013). The role of novel agents on the reversibility of renal impairment in newly diagnosed symptomatic patients with multiple myeloma. *Leukemia*, 27(2), 423–9.
Dimopoulos, M. A., Terpos, E., Chanan-Khan, A., *et al.* (2010c). Renal impairment in patients with multiple myeloma: a consensus statement on behalf of the International Myeloma Working Group. *J Clin Oncol*, 28(33), 4976–84.
Durie, B. G., Harousseau, J. L., Miguel, J. S., *et al.* (2006). International uniform response criteria for multiple myeloma. *Leukemia*, 20(9), 1467–73.
Durie, B. G., Kyle, R. A., Belch, A., *et al.* (2003). Myeloma management guidelines: a consensus report from the Scientific Advisors of the International Myeloma Foundation. *Hematol J*, 4(6), 379–98.
Eleutherakis-Papaiakovou, V., Bamias, A., Gika, D., *et al.* (2007). Renal failure in multiple myeloma: incidence, correlations, and prognostic significance. *Leuk Lymphoma*, 48(2), 337–41.
Endre, Z. H. (2008). Acute kidney injury: definitions and new paradigms. *Adv Chronic Kidney Dis*, 15(3), 213–21.
Endre, Z. H. and Pickering, J. W. (2010). Outcome definitions in non-dialysis intervention and prevention trials in acute kidney injury (AKI). *Nephrol Dial Transplant*, 25(1), 107–18.
Eriksson, T., Hoglund, P., Turesson, I., *et al.* (2003). Pharmacokinetics of thalidomide in patients with impaired renal function and while on and off dialysis. *J Pharm Pharmacol*, 55(12), 1701–6.
Fakhouri, F., Guerraoui, H., Presne, C., *et al.* (2004). Thalidomide in patients with multiple myeloma and renal failure. *Br J Haematol*, 125(1), 96–7.
Guan, S., el-Dahr, S., Dipp, S., *et al.* (1999). Inhibition of Na-K-ATPase activity and gene expression by a myeloma light chain in proximal tubule cells. *J Investig Med*, 47(9), 496–501.
Harris, E., Behrens, J., Samson, D., *et al.* (2003). Use of thalidomide in patients with myeloma and renal failure may be associated with unexplained hyperkalaemia. *Br J Haematol*, 122(1), 160–1.
Healy, J. K. (1963). Acute oliguric renal failure associated with multiple myeloma. Report of three cases. *Br Med J*, 1(5338), 1126–30.
Huang, Z. Q. and Sanders, P.W. (1995). Biochemical interaction between Tamm-Horsfall glycoprotein and Ig light chains in the pathogenesis of cast nephropathy. *Lab Invest*, 73(6), 810–17.
Huang, Z. Q. and Sanders, P. W. (1997). Localization of a single binding site for immunoglobulin light chains on human Tamm-Horsfall glycoprotein. *J Clin Invest*, 99(4), 732–6.
Hutchison, C. A., Batuman, V., Behrens, J., *et al.* (2012a). The pathogenesis and diagnosis of acute kidney injury in multiple myeloma. *Nat Rev Nephrol*, 8(1), 43–51.
Hutchison, C. A., Cockwell, P., Harding, S., *et al.* (2008a). Quantitative assessment of serum and urinary polyclonal free light chains in patients with type II diabetes: an early marker of diabetic kidney disease? *Expert Opin Ther Targets*, 12(6), 667–76.
Hutchison, C. A., Cockwell, P., Reid, S., *et al.* (2007a). Efficient removal of immunoglobulin free light chains by hemodialysis for multiple myeloma: in vitro and in vivo studies. *J Am Soc Nephrol*, 18(3), 886–95.
Hutchison, C. A., Cockwell, P., Stringer, S., *et al.* (2011). Early reduction of serum-free light chains associates with renal recovery in myeloma kidney. *J Am Soc Nephrol*, 22(6), 1129–36.
Hutchison, C. A., Cook, M., Basu, S., *et al.* (2007b). Combined chemotherapy and high cut-off hemodialysis improve outcomes in multiple myeloma patients with severe renal failure. *ASH Annual Meeting Abstracts*, 110(11), 3610.
Hutchison, C. A., Harding, S., Hewins, P., *et al.* (2008b). Quantitative assessment of serum and urinary polyclonal free light chains in patients with chronic kidney disease. *Clin J Am Soc Nephrol*, 3(6), 1684–90.
Hutchison, C. A., Heyne, N., Airia, P., *et al.* (2012b). Immunoglobulin free light chain levels and recovery from myeloma kidney on treatment with chemotherapy and high cut-off haemodialysis. *Nephrol Dial Transplant*, 27(10), 3823–8.
Hutchison, C. A., Plant, T., Drayson, M., *et al.* (2008c). Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. *BMC Nephrol*, 9, 11.
Iggo, N., Palmer, A. B., Severn, A., *et al.* (1989). Chronic dialysis in patients with multiple myeloma and renal failure: a worthwhile treatment. *QJM*, 73(270), 903–10.
Jagannath, S., Barlogie, B., Berenson, J. R., *et al.* (2005). Bortezomib in recurrent and/or refractory multiple myeloma. Initial clinical experience in patients with impared renal function. *Cancer*, 103(6), 1195–200.
Johnson, W. J., Kyle, R. A., and Dahlberg, P. J. (1980). Dialysis in the treatment of multiple myeloma. *Mayo Clin Proc*, 55(2), 65–72.
Johnson, W. J., Kyle, R. A., Pineda, A. A., *et al.* (1990). Treatment of renal failure associated with multiple myeloma. Plasmapheresis, hemodialysis, and chemotherapy. *Arch Intern Med*, 150(4), 863–9.
Kastritis, E., Anagnostopoulos, A., Roussou, M., *et al.* (2007). Reversibility of renal failure in newly diagnosed multiple myeloma patients treated with high dose dexamethasone-containing regimens and the impact of novel agents. *Haematologica*, 92(4), 546–9.
Kastritis, E., Zervas, K., Symeonidis, A., *et al.* (2009). Improved survival of patients with multiple myeloma after the introduction of novel agents and the applicability of the International Staging System (ISS): an analysis of the Greek Myeloma Study Group (GMSG). *Leukemia*, 23(6), 1152–7.
Keeling, J. and Herrera, G. A. (2007). The mesangium as a target for glomerulopathic light and heavy chains: pathogenic considerations in light and heavy chain-mediated glomerular damage. *Contrib Nephrol*, 153, 116–34.
Killmann, S. A., Gjorup, S., and Thaysen, J. H. (1957). Fatal acute renal failure following intravenous pyelography in a patient with multiple myeloma. *Acta Med Scand*, 158(1), 43–6.
Klein, U., Neben, K., Hielscher, T., *et al.* (2010). Lenalidomide in combination with dexamethasone: effective regimen in patients with relapsed or refractory multiple myeloma complicated by renal impairment. *Ann Hematol*, 90(4), 429–39.
Knudsen, L. M., Hjorth, M., and Hippe, E. (2000). Renal failure in multiple myeloma: reversibility and impact on the prognosis. Nordic Myeloma Study Group. *Eur J Haematol*, 65(3), 175–81.
Knudsen, L. M., Nielsen, B., Gimsing, P., *et al.* (2005). Autologous stem cell transplantation in multiple myeloma: outcome in patients with renal failure. *Eur J Haematol*, 75(1), 27–33.
Korzets, A., Tam, F., Russell, G., *et al.* (1990). The role of continuous ambulatory peritoneal dialysis in end-stage renal failure due to multiple myeloma. *Am J Kidney Dis*, 16(3), 216–23.
Kumar, S. K., Rajkumar, S. V., Dispenzieri, A., *et al.* (2008). Improved survival in multiple myeloma and the impact of novel therapies. *Blood*, 111(5), 2516–20.
Kyle, R. A., Gertz, M. A., Witzig, T. E., *et al.* (2003). Review of 1027 patients with newly diagnosed multiple myeloma. *Mayo Clin Proc*, 78(1), 21–33.

Labutti, J., Parsons, I., Huang, R., *et al.* (2006). Oxidative deboronation of the peptide boronic acid proteasome inhibitor bortezomib: contributions from reactive oxygen species in this novel cytochrome P450 reaction. *Chem Res Toxicol*, 19(4), 539–46.

Lee, C. K., Zangari, M., Barlogie, B., *et al.* (2004). Dialysis-dependent renal failure in patients with myeloma can be reversed by high-dose myeloablative therapy and autotransplant. *Bone Marrow Transplant*, 33(8), 823–8.

Leung, N., Bridoux, F., Hutchison, C. A., *et al.* (2012). Monoclonal gammopathy of renal significance: when MGUS is no longer undetermined or insignificant. *Blood*, 120(22), 4292–5.

Leung, N., Gertz, M., Kyle, R. A., *et al.* (2012). Urinary albumin excretion patterns of patients with cast nephropathy and other monoclonal gammopathy-related kidney diseases. *Clin J Am Soc Nephrol*, 7(12), 1964–8.

Ludwig, H., Adam, Z., Hajek, R., *et al.* (2010). Light chain-induced acute renal failure can be reversed by bortezomib-doxorubicin-dexamethasone in multiple myeloma: results of a phase II study. *J Clin Oncol*, 28(30), 4635–41.

Ludwig, H., Drach, J., Graf, H., *et al.* (2007). Reversal of acute renal failure by bortezomib-based chemotherapy in patients with multiple myeloma. *Haematologica*, 92(10), 1411–14.

Ma, C. X., Lacy, M. Q., Rompala, J. F., *et al.* (2004). Acquired Fanconi syndrome is an indolent disorder in the absence of overt multiple myeloma. *Blood*, 104(1), 40–2.

Malpas, J. S. (1969). Management of multiple myeloma. *Br Med J*, 2(5650), 163–5.

Markowitz, G. S., Fine, P. L., Stack, J. I., *et al.* (2003). Toxic acute tubular necrosis following treatment with zoledronate (Zometa). *Kidney Int*, 64(1), 281–9.

Mayo, M. M. and Johns, G. S. (2007). Serum free light chains in the diagnosis and monitoring of patients with plasma cell dyscrasias. *Contrib Nephrol*, 153, 44–65.

Meachem, G. C. and Heinle, R. W. (1950). Association of high ionized serum calcium with renal failure in plasma cell myeloma. *J Clin Invest*, 29(6), 833.

Merlini, G. and Bellotti, V. (2003). Molecular mechanisms of amyloidosis. *N Engl J Med*, 349(6), 583–96.

Merlini, G. and Palladini, G. (2012). Differential diagnosis of monoclonal gammopathy of undetermined significance. *Hematology Am Soc Hematol Educ Program*, 2012, 595–603.

Merlini, G. and Stone, M. J. (2006). Dangerous small B-cell clones. *Blood*, 108(8), 2520–30.

Misiani, R., Tiraboschi, G., Mingardi, G., *et al.* (1987). Management of myeloma kidney: an anti-light-chain approach. *Am J Kidney Dis*, 10(1), 28–33.

Morabito, F., Gentile, M., Ciolli, S., *et al.* (2010). Safety and efficacy of bortezomib-based regimens for multiple myeloma patients with renal impairment: a retrospective study of Italian Myeloma Network GIMEMA. *Eur J Haematol*, 84(3), 223–8.

MRC Working Party on Leukaemia in Adults (1984). Analysis and management of renal failure in fourth MRC myelomatosis trial. *Br Med J (Clin Res Ed)*, 288(6428), 1411–16.

Mulkerin, D., Remick, S., Takimoto, C., *et al.* (2007). Safety, tolerability, and pharmacology of bortezomib in cancer patients with renal failure requiring dialysis: results from a prospective phase 1 Study. *ASH Annual Meeting Abstracts*, 110(11), 3477.

Niesvizky, R., Naib, T., Christos, P. J., *et al.* (2007). Lenalidomide-induced myelosuppression is associated with renal dysfunction: adverse events evaluation of treatment-naive patients undergoing front-line lenalidomide and dexamethasone therapy. *Br J Haematol*, 138(5), 640–3.

Obici, L., Perfetti, V., Palladini, G., *et al.* (2005). Clinical aspects of systemic amyloid diseases. *Biochim Biophys Acta*, 1753(1), 11–22.

Parikh, G. C., Amjad, A. I., Saliba, R. M., *et al.* (2009). Autologous hematopoietic stem cell transplantation may reverse renal failure in patients with multiple myeloma. *Biol Blood Marrow Transplant*, 15(7), 812–16.

Pekol, T., Daniels, J. S., Labutti, J., *et al.* (2005). Human metabolism of the proteasome inhibitor bortezomib: identification of circulating metabolites. *Drug Metab Dispos*, 33(6), 771–7.

Perillie, P. E. and Conn, H. O. (1958). Acute renal failure after intravenous pyelography in plasma cell myeloma. *JAMA*, 167(18), 2186–9.

Pirani, C. L., Valeri, A., D'Agati, V., *et al.* (1987). Renal toxicity of nonsteroidal anti-inflammatory drugs. *Contrib Nephrol*, 55, 159–75.

Pozzi, C., D'Amico, M., Fogazzi, G. B., *et al.* (2003). Light chain deposition disease with renal involvement: clinical characteristics and prognostic factors. *Am J Kidney Dis*, 42(6), 1154–63.

Pozzi, C., Pasquali, S., Donini, U., *et al.* (1987). Prognostic factors and effectiveness of treatment in acute renal failure due to multiple myeloma: a review of 50 cases. Report of the Italien Renal Immunopathology Group. *Clin Nephrol*, 28(1), 1–9.

Reece, D. E., Masih-Khan, E., Chen, C., *et al.* (2006). Use of lenalidomide (Revlimid(R) +/- corticosteroids in relapsed/refractory multiple myeloma patients with elevated baseline serum creatinine levels. *ASH Annual Meeting Abstracts*, 108(11), 3548.

Ronco, P., Plaisier, E., Mougenot, B., *et al.* (2006). Immunoglobulin light (heavy)-chain deposition disease: from molecular medicine to pathophysiology-driven therapy. *Clin J Am Soc Nephrol*, 1(6), 1342–50.

Roussou, M., Kastritis, E., Christoulas, D., *et al.* (2010). Reversibility of renal failure in newly diagnosed patients with multiple myeloma and the role of novel agents. *Leuk Res*, 34(10), 1395–7.

Roussou, M., Kastritis, E., Migkou, M., *et al.* (2008). Treatment of patients with multiple myeloma complicated by renal failure with bortezomib-based regimens. *Leuk Lymphoma*, 49(5), 890–5.

San Miguel, J. F., Lahuerta, J. J., Garcia-Sanz, R., *et al.* (2000). Are myeloma patients with renal failure candidates for autologous stem cell transplantation? *Hematol J*, 1(1), 28–36.

Sanders, P. W., Booker, B. B., Bishop, J. B., *et al.* (1990). Mechanisms of intranephronal proteinaceous cast formation by low molecular weight proteins. *J Clin Invest*, 85(2), 570–6.

Sanders, P. W., Herrera, G. A., and Galla, J. H. (1987). Human Bence Jones protein toxicity in rat proximal tubule epithelium in vivo. *Kidney Int*, 32(6), 851–61.

Sanders, P. W. and Booker, B. B. (1992). Pathobiology of cast nephropathy from human Bence Jones proteins. *J Clin Invest*, 89(2), 630–9.

San-Miguel, J. F., Richardson, P. G., Sonneveld, P., *et al.* (2008). Efficacy and safety of bortezomib in patients with renal impairment: results from the APEX phase 3 study. *Leukemia*, 22(4), 842–9.

Santostefano, M., Zanchelli, F., Zaccaria, A., *et al.* (2005). The ultrastructural basis of renal pathology in monoclonal gammopathies. *J Nephrol*, 18(6), 659–75.

Sarkozi, R., Perco, P., Hochegger, K., *et al.* (2008). Bortezomib-induced survival signals and genes in human proximal tubular cells. *J Pharmacol Exp Ther*, 327(3), 645–56.

Sengul, S., Zwizinski, C., and Batuman, V. (2003). Role of MAPK pathways in light chain-induced cytokine production in human proximal tubule cells. *Am J Physiol Renal Physiol*, 284(6), F1245–54.

Sengul, S., Zwizinski, C., Simon, E. E., *et al.* (2002). Endocytosis of light chains induces cytokines through activation of NF-kappaB in human proximal tubule cells. *Kidney Int*, 62(6), 1977–88.

Shetty, A. and Oreopoulos, D. G. (1995). Continuous ambulatory peritoneal dialysis in end-stage renal disease due to multiple myeloma. *Perit Dial Int*, 15(6), 236–40.

Sirohi, B., Powles, R., Mehta, J., *et al.* (2001). The implication of compromised renal function at presentation in myeloma: similar outcome in patients who receive high-dose therapy: a single-center study of 251 previously untreated patients. *Med Oncol*, 18(1), 39–50.

Solomon, A., Weiss, D. T., and Kattine, A. A. (1991). Nephrotoxic potential of Bence Jones proteins. *N Engl J Med*, 324(26), 1845–51.

Spitzer, T. R., Delmonico, F., Tolkoff-Rubin, N., *et al.* (1999). Combined histocompatibility leukocyte antigen-matched donor bone marrow and renal transplantation for multiple myeloma with end stage renal disease: the induction of allograft tolerance through mixed lymphohematopoietic chimerism. *Transplantation*, 68(4), 480–4.

Spitzer, T. R., Sykes, M., Tolkoff-Rubin, N., *et al.* (2011). Long-term follow-up of recipients of combined human leukocyte antigen-matched bone marrow and kidney transplantation for multiple myeloma with end-stage renal disease. *Transplantation*, 91(6), 672–6.

Terpos, E., Katodritou, E., Tsiftsakis, E., *et al.* (2007). Cystatin-C is a sensitive marker of renal impairment with an independent predictive value for survival in multiple myeloma; reduction post bortezomib monotherapy. *ASH Annual Meeting Abstracts*, 110(11), 1484.

Torra, R., Bladé, J., Cases, A., *et al.* (1995). Patients with multiple myeloma requiring long-term dialysis: presenting features, response to therapy, and outcome in a series of 20 cases. *Br J Haematol*, 91(4), 854–9.

Tosi, P., Zamagni, E., Cellini, C., *et al.* (2004). Thalidomide alone or in combination with dexamethasone in patients with advanced, relapsed or refractory multiple myeloma and renal failure. *Eur J Haematol*, 73(2), 98–103.

Tosi, P., Zamagni, E., Tacchetti, P., *et al.* (2010). Thalidomide-dexamethasone as induction therapy before autologous stem cell transplantation in patients with newly diagnosed multiple myeloma and renal insufficiency. *Biol Blood Marrow Transplant*, 16(8), 1115–21.

Tricot, G., Alberts, D. S., Johnson, C., *et al.* (1996). Safety of autotransplants with high-dose melphalan in renal failure: a pharmacokinetic and toxicity study. *Clin Cancer Res*, 2(6), 947–52.

Tsakiris, D. J., Stel, V. S., Finne, P., *et al.* (2010). Incidence and outcome of patients starting renal replacement therapy for end-stage renal disease due to multiple myeloma or light-chain deposit disease: an ERA-EDTA Registry study. *Nephrol Dial Transplant*, 25(4), 1200–6.

Uttamsingh, V., Lu, C., Miwa, G., *et al.* (2005). Relative contributions of the five major human cytochromes P450, 1A2, 2C9, 2C19, 2D6, and 3A4, to the hepatic metabolism of the proteasome inhibitor bortezomib. *Drug Metab Dispos*, 33(11), 1723–8.

Van Bommel, E. F. (1996). Multiple myeloma treatment in dialysis-dependent patients: to transplant or not to transplant? *Nephrol Dial Transplant*, 11(7), 1486–7.

Vix, V. A. (1966). Intravenous pyelography in multiple myeloma. A review of 52 studies in 40 patients. *Radiology*, 87(5), 896–902.

Walker, F. and Bear, R. A. (1983). Renal transplantation in light-chain multiple myeloma. *Am J Nephrol*, 3(1), 34–7.

Ward, R. A., Greene, T., Hartmann, B., *et al.* (2006). Resistance to intercompartmental mass transfer limits beta2-microglobulin removal by post-dilution hemodiafiltration. *Kidney Int*, 69(8), 1431–7.

Ying, W. Z. and Sanders, P. W. (2001). Mapping the binding domain of immunoglobulin light chains for Tamm-Horsfall protein. *Am J Pathol*, 158(5), 1859–66.

Ying, W. Z., Wang, P. X., Aaron, K. J., *et al.* (2011). Immunoglobulin light chains activate nuclear factor-κB in renal epithelial cells through a Src-dependent mechanism. *Blood*, 117(4), 1301–7.

Zucchelli, P., Pasquali, S., Cagnoli, L., *et al.* (1988). Controlled plasma exchange trial in acute renal failure due to multiple myeloma. *Kidney Int*, 33(6), 1175–80.

CHAPTER 154

Light-chain deposition disease

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Introduction

Light-chain deposition disease (LCDD) is a rare systemic disorder characterized by extracellular tissue deposition of non-amyloid monoclonal immunoglobulin light chains (LCs) in various organs including kidneys, heart, and liver. LCDD occurs often during the course of plasma cell dyscrasias such as multiple myeloma (MM) and amyloidosis or other lymphoproliferative diseases. Idiopathic LCDD in the absence of any detectable haematological disorder is infrequent. Renal involvement in LCDD occurs almost invariably and often leads to renal insufficiency despite aggressive treatment. LCDD recurrence in the renal allograft presents as proteinuria and subsequent progressive graft failure. Median patient survival with LCDD is 49 months, up to 54 months in idiopathic LCDD; death is often due to cachexia, cardiac failure, infections, liver involvement, or progression of MM. While treatment of the underlying lymphoproliferative disease or plasma cell dyscrasia can dramatically alter the clinical course of LCDD in selected patients, novel therapeutic strategies have recently also been successfully applied in patients with idiopathic LCDD.

Aetiology and epidemiology

Approximately 500 mg of free immunoglobulin LCs are produced on a daily basis in normal individuals with twice as much kappa (κ) chains as lambda (λ) chains (Waldmann et al., 1972). Monomeric κ LCs have a half-life of 2–4 hours while dimeric λ LCs have a slightly longer half-life of 3–6 hours (Pratt, 2008). So whilst the production of κ LCs is greater, the serum concentration of the latter is lower under normal circumstances due to the slower clearance of dimeric λ chains resulting in median serum κ levels of 7.3 mg/L (95% range: 3.3–19.4 mg/L) and median λ levels of 12.7 mg/L (95% range: 5.7–26.3 mg/L) with an average κ/λ ratio of approximately 0.50 (Bradwell et al., 2001; Katzmann et al., 2002). Up to 10–30 g of LCs can be metabolized by proximal tubular cells under normal circumstances so baseline serum LC concentrations have to increase to a large extent before the processing capacity of the tubular cells is exceeded (Pratt, 2008). Serum LC concentrations can increase manyfold with progressive degrees of renal failure with a concomitantly increased κ/λ ratio due to the proportionally more extensively reduced clearance of κ LC (Bradwell et al., 2005). The latter phenomenon is also observed with advanced age. A broad range of inflammatory and infectious conditions (e.g. systemic lupus erythematosus) can cause a rise in serum LC concentrations but the κ/λ ratio usually remains normal in these circumstances. A significantly abnormal κ/λ ratio is therefore almost exclusively encountered in the presence of a lymphoproliferative disease with excess production of pathologic LCs and hence disturbance of the normal balance between κ and λ secretion.

LCDD is often associated with MM (varying between 32% and 65% of cases), less frequently with lymphoproliferative disease (lymphoma, chronic lymphatic leukaemia) while in one-third of patients no clear haematological disorder can be identified and LCDD is classified as idiopathic (Lin et al., 2001; Pozzi et al., 2003). In the latter group, the aberrant B-cell population responsible for the pathological LC synthesis is often so small that even sensitive techniques fail to identify the pathological B-cell clone in the bone marrow. Rare associations have been reported together with amyloidosis, diabetic nephropathy, Waldenström macroglobulinaemia, POEMS, crystal-storing histiocytosis, familial Mediterranean fever, and hepatitis C virus infection.

In a large European survey in > 150,000 patients comprising 13 national registries, dialysis treatment for end-stage renal disease (ESRD) due to MM or LCDD had increased in the last 20 years from 0.7 to 2.52 per million population (Tsakiris et al., 2010). The latter observation is probably in part due to better diagnosis and therapy. Mortality risk was 2.77-fold higher in dialysis patients with MM or LCDD while median survival on dialysis was also significantly lower (0.91 years versus 4.46 years) in comparison to patients without these diseases (Tsakiris et al., 2010). In the United States Renal Data System (USRDS) database, immunoproliferative neoplasms (excluding MM, lymphoma, and amyloidosis) were registered as the primary cause of ESRD on 710 occasions between 2004 and 2008, representing 0.1% of all causes (USRDS, 2011). The prevalence of immunoproliferative neoplasms in 2008 was reported as 323 (0.1%) by the USRDS (USRDS, 2011). Although the true prevalence of LCDD is most likely underestimated due to misdiagnosis, it remains a rare disorder.

Pathophysiology

Deposits in LCDD are more frequently of κ LC origin (70%) as opposed to primary amyloidosis (AL) where predominantly λ chains are observed (Lin et al., 2001; Pozzi et al., 2003). Whereas fibrils in AL are usually derived from the variable region of LCs, the granular deposits in LCDD are often constructed of the constant region of the LC with formation of amorphous aggregates with diameters ranging from 50 to 300 nm (Sikkink and Ramirez-Alvarado, 2008). Thermal and chemical denaturation-derived values indicate that LCDD proteins are more stable than AL proteins. Factors potentially affecting the aggregation and deposition of aberrant LCs include somatic mutations, thermodynamic instability, proteolysis, and glycosylation processes and certain germline gene sequences (Sikkink and Ramirez-Alvarado, 2008). The granular extracellular

deposits in LCDD do not form beta-pleated sheets, fibrils, or tubular structures and do not stain with Congo red staining. Organ dysfunction in LCDD is not simply a consequence of LC deposition but also the result of stimulation of various pro-fibrotic pathways leading to expansion of the extracellular matrix (ECM) and fibrosis and ultimately functional organ damage (Keeling and Herrera, 2005, 2009). *In vitro* experiments have demonstrated that renal mesangial cells incubated with LC from LCDD patients, show a significant increase in expression of tenascin, a major protein constituent of mesangial ECM (Keeling and Herrera, 2005, 2009). The increased tenascin concentrations found within the newly formed LC nodules together with a higher expression of platelet-derived growth factor and transforming growth factor beta, indicate a reactive response of the mesangial cells triggered by the presence of LCs and consequently an expansion of the mesangial ECM (Keeling and Herrera, 2005, 2009). Tenascin is degraded by matrix metalloproteinase (MMP)-7 under normal circumstances and to a lesser extent by MMP-3 and MMP-1 (Keeling and Herrera, 2005, 2009). Interestingly, intracellular (vesicles) MMP-7 expression is markedly increased in mesangial cells exposed to LCs while extracellular concentrations of MMP-7 are significantly reduced, suggesting that a defective release of MMP-7 into the ECM might contribute to the mesangial expansion observed in LCDD (Keeling and Herrera, 2005, 2009). In diabetic nephropathy, advanced glycosylation end-products also suppress mesangial cell MMP-7 production, leading to ECM accumulation in a very similar way to that observed in LCDD. Very rarely, an LCDD κ variant is associated with intra-cytoplasmatic crystal deposits in proximal tubular cells giving rise to symptoms of Fanconi syndrome (Larsen et al., 2011). In this latter case, it is thought that filtered κ LCs are in fact resistant to lysosomal degradation within the proximal renal tubular cell because of structural characteristics in their variable region, and accumulate within the lysosome where they undergo spontaneous crystallization. The lack of renal tubular casts in this LCDD variant could be explained by the low affinity of the LC for Tamm–Horsfall proteins and hence low propensity to form casts.

Clinical features

LCDD has an insidious clinical course presenting in the fifth and sixth life decade with a mild predominance for the male gender (~ 60%) (Lin et al., 2001; Pozzi et al., 2003). Many patients develop MM over time and as with amyloidosis, clinical features vary with the location and extent of LC deposition. Typically, patients have cardiac, hepatic, renal, and peripheral neural symptoms. Less frequently, lungs, skin, spleen, adrenal glands, thyroid gland, and gastrointestinal tract are involved (Lin et al., 2001; Pozzi et al., 2003). Rare association have been described with LC deposition in the brain, pharynx, striped muscles, arteries, lymph nodes, and lacrimal glands. Rare cases of LC deposition beneath the basement membrane of the ciliary pigment epithelium leading to exudative retinal detachment and progressive loss of visual acuity have been reported (Daicker et al., 1995). Clinical symptoms and signs are related to the affected organs with hypokinetic cardiomyopathy, cachexia, haemorrhages, infections, and MM progression as main causes of death (Lin et al., 2001; Pozzi et al., 2003). In a survey of 63 patients with LCDD and renal involvement, the incidence death rate was 17.5 per 100 patient-years and median survival was 4.1 years (Pozzi et al., 2003). Factors independently associated with patient survival in this group were age (relative risk (RR) 1.06), the presence of MM (RR 2.75,) and symptomatic extrarenal LC deposition (RR 2.24) (Pozzi et al., 2003). About one-third of patients developed symptomatic extrarenal LC deposition during the course of LCDD. Intensive chemotherapy (vincristine, doxorubicin, dexamethasone (VAD)/vincristine, doxorubicin, methylprednisolone (VAMP)) seemed to improve survival, albeit not significantly. Renal impairment at the time of LCDD diagnosis has also been identified as a significant risk factor for patient death (hazard ratio 2.76) in another study while mean survival was 54 months for patients with LC deposits without associated MM (Lin et al., 2001).

Renal involvement occurs almost invariably in LCDD and dominates the clinical course of the disease as pathological LCs are filtered by the glomerulus and reabsorbed by the proximal tubular cells through receptor-mediated (megalin/cubulin) endocytosis (Keeling and Herrera, 2005, 2009). LCs undergo degradation in the renal tubular cells by lysosomal enzymes such as pepsin and cathepsin B. Depending on their structural characteristics, LC can either form casts with Tamm–Horsfall proteins in the distal tubules or not, depending on their specific binding properties. Rarely the LCs, usually κ chains, crystallize in the cytoplasm of the proximal tubular cells (Larsen et al., 2011). More than 90% of patients with LCDD have renal functional impairment at the time of diagnosis. In 30–40% of cases they present clinically with acute or rapidly progressive kidney failure (usually developing over a period of a few months) or patients are diagnosed with chronic renal insufficiency as evidenced by pre-existing abnormal laboratory values with or without the presence of reduced kidney size (Lin et al., 2001; Pozzi et al., 2003; Stratta et al., 2011). Patients with idiopathic LCDD less often present with acute kidney failure and have lower median serum creatinine concentrations compared to patients with concurrent MM (Lin et al., 2001; Pozzi et al., 2003; Stratta et al., 2011). The incidence rate of ESRD was 23.7 per 100 patient-years while the median time to reach ESRD was 2.7 years in a cohort of 63 LCDD patients (Pozzi et al., 2003). Age (RR 1.05) and serum creatinine at presentation (RR 1.24) were independently associated with renal survival (Pozzi et al., 2003). Interestingly, no histological features in the renal biopsy were predictive of ESRD in this clinical cohort (Pozzi et al., 2003). Nephrotic range proteinuria is present in 40–50% of patients with LCDD at the time of diagnosis; approximately 20% of patients develop a full-blown clinical nephrotic syndrome. Forty-five per cent of patients have a moderate degree of proteinuria at presentation ranging between 1 and 3.5 g/24 hours. Other nephrological signs at presentation are arterial hypertension (80%), oedema (40–60%), and microscopic haematuria (40–50%) (Lin et al., 2001; Pozzi et al., 2003; Stratta et al., 2011).

Histopathology

A typical renal biopsy in a patient with LCDD contains enlarged glomeruli with nodular or diffuse expansion of the mesangial matrix, often accompanied by mild mesangial hypercellularity (Fig. 154.1A). Occasionally, membranoproliferative features with mesangial interposition are noticed around mesangial nodules. The nodular depositions stain periodic acid–Schiff (PAS)-positive. Glomerulosclerosis is common but variable while glomerular and tubular basement membrane thickening is frequently observed (80% of cases). The light microscopic picture of a nodular sclerosing type of glomerulopathy is encountered in 50% of LCDD cases

(Lin et al., 2001; Pozzi et al., 2003). Sometimes only very minimal changes are observed on light microscopic examination. In the tubulointerstitial compartment, the concurrent damage varies from mild to severe tubular atrophy and interstitial fibrosis. Histological evidence of associated myeloma cast nephropathy is regularly encountered in patients with LCDD and MM. Associated cases of amyloidosis are less frequent observed and can be differentiated by Congo red staining and electron microscopy (EM). Other histological entities in light microscopic examinations of renal biopsies of LCDD patients have been described, including heavy-chain deposition disease, diabetic nephropathy, fibrillary glomerulopathy, thrombotic microangiopathy, acute tubulointerstitial nephritis, and proximal tubular LC crystal deposition. Immunohistochemistry with monoclonal antibodies against κ and λ LC (immunofluorescence or immunostaining) is positive in > 95% of patients with staining of the glomerular nodules, glomerular basement membrane, tubular basement membrane, and vascular walls in 40%, 82%, 95%, and 63% respectively (Lin et al., 2001; Pozzi et al., 2003). κ is the dominant LC found in LCDD renal biopsies and is more often associated with a nodular sclerosing glomerulopathy type compared to λ chain LCDD (Fig. 154.1C, D). EM demonstrates granular electron-dense deposits along the glomerular and tubular basement membrane (lamina rara interna) and less frequently along the vascular wall (in the basement membrane of individual myocytes) (Fig. 154.1B). Interstitial deposits are rare. Fusion of podocyte foot processes is variably identified but typically extensive. In approximately 15–20% of cases, EM fails to confirm the presence of electron-dense deposits in a renal biopsy with positive immunohistochemistry for κ or λ chains (Lin et al., 2001). The latter occurs more frequently in patients with LCDD in combination with MM and more with λ chain LCDD. The discrepancy between IF and EM findings in LCDD can possibly be explained by differences in LC structure and hence altered propensity for the formation of electron-dense deposits and the often focal distribution of deposits. Conversely, typical granular electron-dense deposits are only rarely found on EM in LCDD biopsies with negative immunohistochemistry (Lin et al., 2001). This lack of immune reactivity may be explained by structural alterations in the antigenic properties of the LC that lead to the obscuring of epitopes for antibody recognition.

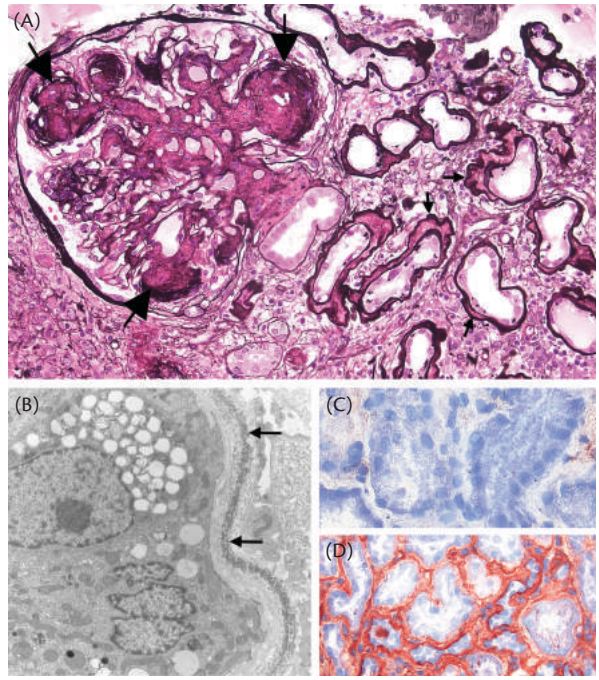

Fig. 154.1 Light microscopy, electron microscopy, and immunostaining of a native renal biopsy containing idiopathic LCDD. (A) Silver methenamine stain (original magnification ×200). Large arrows indicate the presence of argyrophilic nodular mesangial expansions resembling Kimmelstiel–Wilson nodules in diabetic nephropathy. Small arrows show the thickening and sometimes reduplication of the tubular basement membranes. (B) Transmission electron microscopy (original magnification ×4400). Arrows indicate the presence of electron-dense granular deposits along the tubular basement membrane. (C) Immunohistochemical stain on frozen tissue using antibodies against λ LCs (original magnification ×200), showing the absence of λ LCs along the tubular basement membranes. (D) Immune-histochemical stain on frozen tissue using antibodies against κ LCs (original magnification ×200), showing diffuse and strong positivity for κ LCs along the tubular basement membranes.
Courtesy of Prof Dr Evelyne Lerut, Department of Morphology and Molecular Pathology, University Hospitals of Leuven, Belgium.

Diagnostic approach and differential diagnosis

The diagnosis of LCDD is often delayed and based on the insidious clinical presentation which is strongly dependent on the localization of LC deposition(s), the almost invariable renal involvement, and the detection of an excess of abnormal immunoglobulin LCs. Whilst bone marrow examination will often identify associated MM, renal biopsy frequently provides the final diagnostic proof, especially in cases of idiopathic LCDD. Different types of diagnostic imaging techniques can be employed to document and semi-quantify extrarenal LC deposition including computed tomography (CT), ultrasound, magnetic resonance imaging, and recently F-18 FDG positron emission tomography/CT (Makis et al., 2010).

Abnormal LCs can be detected and quantified by serum or urine protein electrophoresis and immunofixation and serial measurements are helpful for disease monitoring and therapeutic response evaluation (Wolff et al., 2007). By immunofixation a monoclonal protein can be detected in the serum in approximately 75% of LCDD patients and in 90% of cases in the urine (Wolff et al., 2007). Only in a small group of patients with LCDD (< 10%) can no pathological LC be demonstrated by employing this analytical method. The diagnostic value of serum or urine protein electrophoresis in the detection of LCDD is poor with only one-third of patients having a 'M protein' or 'M spike' detected prior to histological confirmation of the disease (Wolff et al., 2007). The recently developed automated immunoassay (Freelite™, The Binding Site Ltd.) with nephelometric detection and quantification of free κ and λ LCs in serum and urine, has been accepted as a screening tool and an additive or alternative clinically prognostic and monitoring tool, especially in the management of oligo-secretory MM and other oligo-secretory plasma cell disorders (guidelines of the International Myeloma Working Group) (Pratt, 2008; Dispenzieri et al., 2009). No large validation studies of this quantitative nephelometric assay in LCDD patients have been reported. Based on the sparse data extracted from mixed population studies, the use of the free LC immunoassay might prove beneficial in the diagnosis and therapeutic monitoring of certain cases of LCDD but further validation is mandatory (Tate et al., 2003; Katzmann et al., 2005; Pratt,

2008; Briand et al., 2010). For example, the intrinsic limitations of the free LC immunoassay including poor post-dilution linearity, overestimation by nephelometry, the variation in results with different analysers, and the need for local reference ranges, have not been appropriately addressed in the clinical setting of disease management of LCDD (Tate et al., 2003; Katzmann et al., 2005; Pratt, 2008; Briand et al., 2010).

In the differential diagnosis, LCDD should be clinically differentiated from AL and secondary (AA) amyloidosis, MM, and other forms of plasma cell dyscrasias.

Treatment and outcome

By definition, all patients who have LCDD have a clonal population of bone marrow plasma cells synthesizing the LCs or heavy chains or fragments of either that ultimately form granular deposits in the kidney visible by EM. Monoclonal immunoglobulin deposition disease due to amyloid, a fibrillar rather than glomerular deposition, and myeloma cast nephropathy, where the immunoglobulin LC precipitates with Tamm–Horsfall glycoprotein in the renal tubule, have different mechanisms. All share the same therapeutic strategy whereby eradication of LC synthesis is required both to result in disease regression and to prevent disease recurrence if renal allografting is undertaken.

One indication of the common pathology of all monoclonal immunoglobulin deposition disorders are reports of renal failure due to combined cast nephropathy, amyloidosis, and LCDD (Lorenz et al., 2010). The coexistence of myeloma cast nephropathy, LCDD, and non-amyloid fibrils in a patient with MM has also been reported, suggesting a common underlying mechanism for cast nephropathy and the deposition of LCs in the kidney. Oftentimes, distinction between these requires laser microdissection and mass spectrometry (Qian et al., 2010).

Therapy directed against LC synthesis is not required in all patients. In the majority of patients, the disease throughout its clinical course involves only the kidneys. Case reports of LCDD involving the heart and liver exist, but these are rare and the risk of development of extrarenal LC deposition does not mandate therapy if no other indications exist.

Patients can present with LCDD in oliguric renal failure. In this situation, chemotherapy is unlikely to restore normal renal function; and if renal transplantation is not anticipated, no chemotherapy is required and renal replacement therapy can commence. Dialysis does not seem to worsen the outcome in LCDD because survival of patients on dialysis is not different from the other patients not reaching uraemia (Pozzi and Locatelli, 2002). Therefore, if a renal transplant is not planned and there is no evidence of extrarenal deposition of LCs or if comorbidities render the patient a poor candidate for chemotherapy, no intervention for patients who have already achieved dialysis dependency is required.

For patients that are candidates for active management, cytotoxic chemotherapy directed against the monoclonal immunoglobulin-producing plasma cells is indicated. The recent introduction of immunoglobulin free LC measurement has resulted in a sensitive nephelometric test that renders it possible to monitor the actual synthesis of the responsible monoclonal immunoglobulin and assess for response, as evidenced by a decline in LC production, or evidence of disease recurrence with a rise in LCs. The ratio of involved and uninvolved immunoglobulin LCs allows monitoring to occur even in those patients who have significant renal insufficiency, which reduces the glomerular excretion of LCs (Brockhurst et al., 2005a, 2005b). It has been reported that improvement in renal function and proteinuria following chemotherapy correlates with a reduction in circulating LC levels, and this has been our experience as well.

Renal transplantation is an appropriate consideration for patients with LCDD; but in the absence of cytotoxic chemotherapy to suppress immunoglobulin LC production, recurrence of the disease after renal allografting is to be expected. One patient with LCDD lost her first living donor graft at 1 year due to extensive recurrence of κ LC deposition. Rituximab therapy prevented loss of a second graft, but there was mild recurrence of LC deposits (Kuypers et al., 2007). Others have also reported LCDD recurring after renal transplantation (Larsen et al., 2008; Taneda et al., 2008). The most common causes of death in LCDD patients are cardiovascular causes, malignancy, and infection. There was a 2.77-fold higher risk of death compared to other patients without LC deposition. An unadjusted median survival of patients on renal replacement therapy was 0.91 years. Results suggest that dialysis should be offered to patients with LCDD (Tsakiris et al., 2010).

The treatment of LCDD has paralleled treatment of myeloma cast nephropathy. The first report of multiple alkylating agent chemotherapy for LCDD appeared in the year 2000 where long-term intermittent melphalan, cyclophosphamide, vincristine, and prednisolone were administered to a 64-year-old man with LC nephropathy and nodular deposits of κ LCs in the mesangium and subendothelial space. Proteinuria and renal function improved. A follow-up biopsy showed diminution of nodular lesions and disappearance of κ LC deposits validating the value of chemotherapy (Tsakiris et al., 2010). A 37-year-old male with nephrotic syndrome and renal insufficiency was treated with melphalan and steroids, and the LC protein disappeared from the serum and urine with stable renal function for > 10 years. A nephrectomy performed for renal cell cancer demonstrated that the nodular lesions and LC deposits were no longer observed (Komatsuda et al., 2000). A 58-year-old female who had proteinuria and a creatinine clearance of 49 mL/min had three courses of melphalan and prednisone chemotherapy resulting in disappearance of proteinuria and stabilization of renal function, demonstrating that early systemic chemotherapy can be beneficial in this patient population (Hotta and Taguma, 2002).

The past decade has resulted in dramatic improvements in the efficacy of systemic chemotherapy available for these patients. Since the outcome of the disease is driven primarily by eradication of the monoclonal immunoglobulin, deep reductions in LC synthesis are required. This is not the case for MM where modest reductions of the tumour mass can result in prolonged survival. In immunoglobulin deposition, 50% reduction of LC production may have no impact on the inexorable progression of LC deposition into the renal parenchyma. In the last 15 years, however, the introduction of immunomodulatory drugs, proteasome inhibitors, and stem cell transplantation have improved the outlook for these patients.

A 32-year-old female with LCDD who presented with nephrotic syndrome received prednisolone and cytotoxic chemotherapy as used for MM, and it was ineffective. Thalidomide 100 mg daily and dexamethasone 20 mg, 4 days per month resulted in a complete haematologic remission after 8 months, including a normalized free LC ratio, and improvement of the renal insufficiency. At the

time of the report, the response was 31 months and continuing (Nakatsuka et al., 2005).

Bortezomib is a highly active anti-myeloma agent approved for use in patients with newly diagnosed and relapsed refractory myeloma. It has the particular advantage that no dose modification is required for renal insufficiency. Multiple reports of bortezomib ability to reverse renal failure in MM exist. In the last 5 years, phase I and II studies have shown it to be highly effective, producing both haematologic and organ responses in amyloidosis.

A 39-year-old male who had failed two chemotherapy regimens and stem cell transplantation developed renal failure. At 3 days following living donor renal transplantation, he displayed rapid deterioration of renal function that failed to respond to antirejection therapy. A renal biopsy showed recurrent LCDD with immunofluorescent staining of the basement membranes, and vessels for κ LCs. The LCs were also detected in the serum and urine. Following suspension of sirolimus given to prevent graft rejection, he was initiated on bortezomib and dialysis was discontinued within 3 weeks with progressively improving renal function. Maintenance therapy consisted of six 2-week-long cycles of bortezomib separated by a 1-week rest. The bortezomib rescue therapy salvaged the kidney with pathologically proven recurrent LCDD (Fujita et al., 2011). A patient whose kidney biopsy revealed κ LC nephropathy with PAS-negative tubular casts in the cortex and outer medulla was reported, with 15% κ plasma cells in the bone marrow. The patient received a 3-day course of plasma exchange followed by eight cycles of bortezomib, liposomal pegylated doxorubicin, and dexamethasone and did not require dialysis. Cyclophosphamide was added, as was thalidomide maintenance, and 32 months after diagnosis, the patient's renal function was improved and he achieved a partial response. Bortezomib-based chemotherapy is effective and should be considered in the treatment of LCDD with severe renal dysfunction.

Stem cell transplantation

Stem cell transplantation is uniquely suited to the management of monoclonal immunoglobulin deposition diseases. Unlike MM, the plasma cell burden in LCDD is low, usually 5% or less clonal plasma cells in the bone marrow. In addition, the cells do not have a high prevalence of genetic abnormalities and are non-proliferative so that a single course of high-dose chemotherapy can result in long-term suppression of plasma cell proliferation and thereby eliminate LC deposition over long periods of time. The first report of dialysis-dependent renal failure and LCDD managed by autologous stem cell transplantation was reported in 2004. This patient was dialysis dependent and treatment with autologous stem cell transplantation led to reversal and sustained improvement in renal function (Kaposztas et al., 2009). A 53-year-old male with nephrotic syndrome and severe renal failure was diagnosed with light- and heavy-chain deposition disease by renal biopsy. Conventional chemotherapy was ineffective and did not improve renal failure. High-dose chemotherapy with peripheral blood stem cell transplantation was introduced after haemodialysis and normalized the bone marrow with improved kidney function (Firkin et al., 2004).

A group at Boston University Medical Center reported five patients with LCDD who received high-dose melphalan and autologous peripheral blood stem cell transplantation. Five of the six were predominantly renal, and one patient had biopsy-proven deposits in the myocardium. All patients were alive and five of the six achieved a complete haematologic remission at a median follow-up of 12 months after stem cell transplantation, indicating the feasibility and effectiveness of this treatment approach (Sakakima et al., 2005). Sequential autologous peripheral blood stem cell transplantation and kidney transplantation has been utilized in LCDD (Weichman et al., 2006). There is controversy as to whether it is appropriate to perform a stem cell transplantation followed by a renal transplant or whether a renal transplant should precede a stem cell transplant. There are advantages and disadvantages to both techniques. If the patient receives high-dose chemotherapy in the face of significant degrees of renal impairment, the dose must be reduced by 30% to account for the renal insufficiency. In this circumstance, the reduced dose will likely reduce the response rate. As a consequence, these patients may undergo stem cell transplantation, have residual disease, and not be ideal candidates for a renal transplant because of the high risk of recurrence. If the patient receives a kidney transplant first and then undergoes high-dose chemotherapy at full dose and they do not achieve a complete response, one questions the wisdom of the decision to allocate a kidney given the risk of recurrence. The controversy regarding sequencing of stem cell and renal transplant continues and is not yet resolved.

Seven patients were reported with monoclonal immunoglobulin deposition disease. Their median age was 50, six were male. A κ LC was detected by the serum immunoglobulin free LC assay in all. The patients received a melphalan 140 mg/m^2 auto-transplant. All patients were alive with six in haematologic complete response with a median follow-up of 23.6 months. Renal function improved compared to the pre-transplant creatinine in five. Two subsequently had a renal transplant and became dialysis independent with one stable and one worsening leading to resumption of dialysis despite a haematologic complete response. The authors argue in favour of kidney transplantation in those patients who achieved haematologic complete response, which fits with our own philosophy (Barraclough et al., 2007). One patient with severe renal dysfunction due to LCDD received high-dose melphalan and stem cell transplant that extended the period of improved renal function. Four years after the initial improvement, the patient developed nephrotic range proteinuria; and at that time, a renal biopsy showed complete resolution of LC deposition and development of extensive glomerulosclerosis accounting for the proteinuria. This was the first report of biopsy-proven resolution of renal LCDD following autologous stem cell transplant (Hassoun et al., 2008). A 55-year-old female presented with heavy proteinuria (6.2 g/day). An IgG κ monoclonal protein was detected in the serum and urine. The bone marrow showed 6% plasma cells. Renal biopsy showed positive staining for κ LCs in nodular lesions, proximal tubules, and Bowman's capsules. The proteinuria disappeared and renal function stabilized after high-dose chemotherapy and stem cell transplantation (Petrakis et al., 2010).

Our own experience with stem cell transplant and LCDD mirrors the previously reported successes. We have transplanted six patients with LCDD. The patients received dexamethasone alone, dexamethasone plus thalidomide, or no chemotherapy prior to transplant. All received high-dose melphalan. Four were male; their median age was 43.5 years, and the median serum creatinine was 2.4 mg/dL with a median estimated glomerular filtration rate (eGFR) of 26.5 mL/min. One patient died day 26 post transplant. The median follow-up of the five survivors is 31.7 months, all achieved a haematologic response, although

two ultimately relapsed and required further chemotherapy. The eGFR of one patient declined at the time of relapse and improved with subsequent therapy. The eGFR of the second patient was stable throughout relapse and treatment. The patient on haemodialysis prior to transplant continued to require dialysis but subsequently received a renal transplant. The median reduction in proteinuria was 92%, and median improvement in eGFR was 95%. Of four evaluable patients, all achieved criteria for a renal response. We believe that stem cell transplant is an effective therapy and that those patients who achieve a complete hematologic response become candidates for renal transplantation (Matsuzaki et al., 2011).

Renal transplantation

Renal allografting without chemotherapy inevitably results in disease recurrence in the graft. Patients in whom renal deposition of LCs is associated with proliferative glomerulonephritis have a worse graft survival than those presenting with cast nephropathy (Lorenz et al., 2008). We have performed renal allografting in seven patients with LCDD. Renal insufficiency, hypertension, and proteinuria were present in all seven. Proteinuria was > 3.5 g/day in three. Monoclonal protein was detectable in the urine in five. Median age at presentation was 43 years. A κ LC was detected in all seven renal biopsies. Five were on dialysis prior to transplantation. LCDD recurred after a median of 33 months in five of the seven patients. One remains on dialysis; the other four have died. Only one patient was recurrence-free 13 years after transplant with normal function. Kidney transplantation without aggressive chemotherapy to eliminate LC production should not be considered.

One patient with λ LCDD has been reported, who received cadaveric renal transplant with recurrent disease (Short et al., 2001). Investigations are underway using non-chemotherapy approaches for suppressing immunoglobulin free LC production. RNA interference to inhibit LC synthesis has been investigated in a mouse myeloma model. A myeloma cell line producing λ2 immunoglobulin LCs were treated with small iRNAs directed specifically to the V, J, or C portions of the molecules. Transfected cells express detectable quantities of messenger RNA; and after exposure to small interfering RNAs, a 40% reduction in LC production was evidenced at 48 hours. RNA interference can markedly reduce LC synthesis and provide the basis for testing this strategy in *in vivo* models of LC monoclonal immunoglobulin protein deposition disorders.

In summary, chemotherapy is highly effective. Bortezomib, reported on a limited basis, appears to be a highly active agent. Stem cell transplant appears to have the longest track record in the management of this disorder and is recommended for those patients either to preserve renal function or for achieving complete haematologic response in preparation for renal allografting.

Acknowledgement

We thank Prof Dr Evelyne Lerut for providing the microphotographs for Fig. 154.1.

References

Barraclough, K. A., Dowling, J. P., Schwarer, A. P., *et al.* (2007). Sequential autologous peripheral blood stem cell transplantation and kidney transplantation of light chain deposition disease. *Nephrol Dial Transplant*, 22, 1268–9.

Bradwell, A. R., Carr-Smith, H. D., Mead, G. P., *et al.* (2001). Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin Chem*, 47, 673–80.

Bradwell, A. R., Evans, E. D., Chappell, M. J., *et al.* (2005). Rapid removal of free light chains from serum by hemodialysis for patients with myeloma kidney. *Blood*, 106, 972a.

Briand, P. Y., Decaux, O., Caillon, H., *et al.* (2010). Analytical performance of the serum free light chain assay. *Clin Chem Lab Med*, 48(1), 73–9.

Brockhurst, I., Harris, K. P. G., and Chapman, C. S. (2005a). Diagnosis and monitoring a case of light-chain deposition disease in the kidney using a new, sensitive immunoassay. *Nephrol Dial Transplant*, 20, 1251–3.

Brockhurst, I., Harris, K. P. G., and Chapman, C. S. (2005b). Erratum: Diagnosis and monitoring a case of light chain deposition disease in the kidney using a new, sensitive immunoassay (*Nephrology Dialysis Transplantation* (2005) vol. 20 (1251–1253)). *Nephrol Dial Transplant*, 20, 2584.

Daicker, B. C., Mihatsch, M. J., Strom, E. H., *et al.* (1995). Ocular pathology in light chain deposition disease. *Eur J Ophthalmol*, 5(2), 75–81.

Dispenzieri, A., Kyle, R., Merlini, G., *et al.* (2009). International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukemia*, 23, 215–24.

Firkin, F., Hill, P. A., Dwyer, K., *et al.* (2004). Reversal of dialysis-dependent renal failure in light-chain deposition disease by autologous peripheral blood stem cell transplantation. *Am J Kidney Dis*, 44, 551–5.

Fujita, H., Hishizawa, M., Sakamoto, S., *et al.* (2011). Durable hematological response and improvement of nephrotic syndrome on thalidomide therapy in a patient with refractory light chain deposition disease. *Int J Hematol*, 93(5), 673–6.

Hassoun, H., Flombaum, C., D'Agati, V. D., *et al.* (2008). High-dose melphalan and auto-SCT in patients with monoclonal Ig deposition disease. *Bone Marrow Transplant*, 42, 405–12.

Hotta, O. and Taguma, Y. (2002). Resolution of nodular glomerular lesions in a patient with light-chain nephropathy. *Nephron*, 91, 504–5.

Kaposztas, Z., Kahan, B. D., Katz, S. M., *et al.* (2009). Bortezomib successfully reverses early recurrence of light-chain deposition disease in a renal allograft: a case report. *Transplant Proc*, 41, 4407–10.

Katzmann, J. A., Abraham, R. S., Dispenzieri, A., *et al.* (2005). Diagnostic performance of quantitative κ and λ free light chain assays in clinical practice. *Clin Chem*, 51(5), 878–81.

Katzmann, J. A., Clark, R. J., Abraham, R. S., *et al.* (2002). Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. *Clin Chem*, 48, 1437–44.

Keeling, J. and Herrera, G. A. (2005). Matrix metalloproteinases and mesangial remodeling in light chain-related glomerular damage. *Kidney Int*, 68, 1590–603.

Keeling, J. and Herrera, G. A. (2009). An in vitro model of light chain deposition disease. *Kidney Int*, 75, 634–45.

Komatsuda, A., Wakui, H., Ohtani, H., *et al.* (2000). Disappearance of nodular mesangial lesions in a patient with light chain nephropathy after long-term chemotherapy. *Am J Kidney Dis*, 35, E9.

Kuypers, D. R. J., Lerut, E., Claes, K., *et al.* (2007). Recurrence of light chain deposit disease after renal allograft transplantation: Potential role of rituximab? *Transplant Int*, 20, 381–5.

Larsen, C. P., Bell, J. M., Harris, A. A., *et al.* (2011). The morphologic spectrum and clinical significance of light chain proximal tubulopathy with and without crystal formation. *Mod Pathol*, 24(11), 1462–9.

Larsen, T., Hammer, A., and Jorgensen, K. A. (2008). Recurrence of light-chain deposition disease after renal transplantation. *Scand J Urol Nephrol*, 42, 187–8.

Lin, J., Markowitz, G. S., Valeri, A. M., *et al.* (2001). Renal monoclonal immunoglobulin deposition disease: the disease spectrum. *J Am Soc Nephrol*, 12, 1482–92.

Lorenz, E. C., Gertz, M. A., Fervenza, F. C., *et al.* (2008). Long-term outcome of autologous stem cell transplantation in light chain deposition disease. *Nephrol Dial Transplant*, 23, 2052–7.

Lorenz, E. C., Sethi, S., Poshusta, T. L., *et al.* (2010). Renal failure due to combined cast nephropathy, amyloidosis and light-chain deposition disease. *Nephrol Dial Transplant*, 25, 1340–3.

Makis, W., Derbekyan, V., and Novales-Diaz, J. A. (2010). Pulmonary light and heavy chain deposition disease. A pitfall for lung cancer evaluation with F-18 FDG PET/CT. *Clin Nucl Med*, 35(8), 640–3.

Matsuzaki, K., Ohsawa, I., Nishitani, T., *et al.* (2011). Marked improvement by high-dose chemotherapy and autologous stem cell transplantation in a case of light chain deposition disease. *J Nephrol*, 24, 246–9.

Nakatsuka, A., Maeshima, Y., Sarai, A., *et al.* (2005). A case of monoclonal immunoglobulin light- and heavy-chain deposition disease exhibiting atypical deposition with fibrillary structures, successfully treated with chemotherapy. *Clin Nephrol*, 64, 221–7.

Petrakis, I., Stylianou, K., Mavroeidi, V., *et al.* (2010). Biopsy-proven resolution of renal light-chain deposition disease after autologous stem cell transplantation. *Nephrol Dial Transplant*, 25, 2020–3.

Pozzi, C., D'Amico, M., Fogazzi, G. B., *et al.* (2003). Light chain deposition disease with renal involvement: clinical characteristics and prognostic factors. *Am J Kidney Dis*, 42, 1154–63.

Pozzi, C. and Locatelli, F. (2002). Kidney and liver involvement in monoclonal light chain disorders. *Semin Nephrol*, 22, 319–30.

Pratt, G. (2008). The evolving use of serum free light chain assays in haematology. *Br J Haematol*, 141, 413–22.

Qian, Q., Leung, N., Theis, J. D., *et al.* (2010). Coexistence of myeloma cast nephropathy, light chain deposition disease, and nonamyloid fibrils in a patient with multiple myeloma. *Am J Kidney Dis*, 56, 971–6.

Sakakima, M., Fujigaki, Y., Tsuji, T., *et al.* (2005). High dose chemotherapy and stem cell support in a patient of light- and heavy-chain deposition disease with abnormal marrow cell surface antigens and no monoclonal protein. *Intern Med*, 44, 970–4.

Short, A. K., O'Donoghue, D. J., Riad, H. N., *et al.* (2001). Recurrence of light chain nephropathy in a renal allograft. A case report and review of the literature. *Am J Nephrol*, 21, 237–40.

Sikkink, L. A. and Ramirez-Alvarado, M. (2008). Biochemical and aggregation analysis of Bence Jones proteins from different light chain diseases. *Amyloid*, 15(1), 29–30.

Stratta, P., Gravellone, L., Cena, T., *et al.* (2011). Renal outcome and monoclonal immunoglobulin deposition disease in 289 old patients with blood cell dyscrasias: a single center experience. *Crit Rev Oncol Hematol*, 79, 31–42.

Taneda, S., Honda, K., Horita, S., *et al.* (2008). Light chain deposition disease after renal transplantation. *Am J Kidney Dis*, 52, 621–5.

Tate, J. R., Gill, D., Cobcroft, R., *et al.* (2003). Practical considerations for the measurement of free light chains in serum. *Clin Chem*, 49(8), 1252–7.

Tsakiris, D. J., Stel, V. S., Finne, P., *et al.* (2010). Incidence and outcome of patients starting renal replacement therapy for end-stage renal disease due to multiple myeloma or light-chain deposit disease: an ERA-EDTA Registry Study. *Nephrol Dial Transplant*, 25, 1200–6.

United States Renal Data System (2011). *USRDS Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States*. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases.

Waldmann, T. A., Strober, W., and Mogielnicki, R. P. (1972). The renal handling of low molecular weight proteins. II. Disorders of serum protein catabolism in patients with tubular proteinuria, the nephrotic syndrome, or uremia. *J Clin Invest*, 51, 2162–74.

Weichman, K., Dember, L. M., Prokaeva, T., *et al.* (2006). Clinical and molecular characteristics of patients with non-amyloid light chain deposition disorders, and outcome following treatment with high-dose melphalan and autologous stem cell transplantation. *Bone Marrow Transplant*, 38, 339–43.

Wolff, F., Thiry, C., and Willems, D. (2007). Assessment of the analytical performance and the sensitivity of serum free light chains immunoassay in patients with monoclonal gammopathy. *Clin Biochem*, 40, 351–4.

CHAPTER 155

Other consequences from monoclonal immunoglobulins/fragments: membranoproliferative glomerulonephritis and acquired Fanconi syndrome

Fernando C. Fervenza, S. Vincent Rajkumar, and Sanjeev Sethi

Introduction

Renal damage secondary to deposition of monoclonal immunoglobulin can occur due to accumulation of either light chains, heavy chains, or both (Dhar et al., 1977; Alpers et al., 1985; Rosenstock et al., 2003; Touchard et al., 2003). These include myeloma kidney (cast nephropathy), light-chain (AL) and heavy-chain (AH) amyloidosis, and light- and heavy-chain deposition disease (Alpers et al., 1985; Rosenstock et al., 2003; Leung and Rajkumar, 2007). Renal damage secondary to deposition of both chains is far less common (Sanders, 1993; Rosenstock et al., 2003; Touchard et al., 2003). In the great majority of these cases the M-component is immunoglobulin (Ig)-G (Kebler et al., 1985; Fakhouri et al., 2002; Nasr et al., 20040), but the spectrum of renal lesions associated with monoclonal gammopathy is extensive and depends on the physicochemical properties of the immunoglobulin produced (Audard et al., 2008). Cases of Waldenström macroglobulinaemia causing renal disease due to monoclonal deposits are also described, but it is less common than in multiple myeloma (MM) and the monoclonal deposits usually have a IgM component (Veltman et al., 1997). In this chapter, we discuss two important, but less recognized disorders associated with monoclonal immunoglobulins: membranoproliferative glomerulonephritis (MPGN) and acquired Fanconi syndrome.

Membranoproliferative glomerulonephritis secondary to intact monoclonal immunoglobulin deposition

While light-chain deposition disease is a well-known entity, MPGN secondary to intact monoclonal immunoglobulin deposition is less frequently recognized (Masai et al., 2009). As described below, monoclonal gammopathies may be causally related to a significant proportion of idiopathic MPGN in adults, making this an important clinical consideration, with implications for therapy and prognosis.

Pathology and clinical considerations

We recently analysed renal biopsies of patients diagnosed with idiopathic MPGN at the Mayo Clinic over a 6-year period from 2001 to 2006 (Sethi et al., 2011). Among the 65 patients with hepatitis-negative, lupus-negative MPGN, 28 patients (43.1%) were positive for monoclonal/biclonal immunoglobulins. Light microscopic examination of renal biopsies showed an MPGN pattern of injury with glomeruli showing an expanded mesangium with an increase in matrix material and cellularity (Fig. 155.1A, B). Glomerular basement membranes were thickened, and many capillary loops showed subendothelial expansion with cellular elements, eosinophilic deposits, and new basement membrane formation resulting in double contours. Immunofluorescence (IF) microscopy showed granular immune deposits in the mesangium and/or along the capillary walls, consisting of IgM kappa = 11, IgG kappa = 4, IgG (heavy chain only) = 2, IgG lambda = 1, IgM lambda = 1, and IgG/IgM lambda = 1 case (Fig. 155.1C–E). These results correlated with immunofixation electrophoresis results (in two cases IgG was noted in the mesangium and along capillary walls, but light-chain restriction was not documented). In most biopsies, the deposits were more prominent along the capillary walls than in the mesangium, while in few others the reverse was true. On electron microscopy (EM) examination there was thickening of the capillary walls with subendothelial deposits in all cases (Fig. 155.1F). Cellular

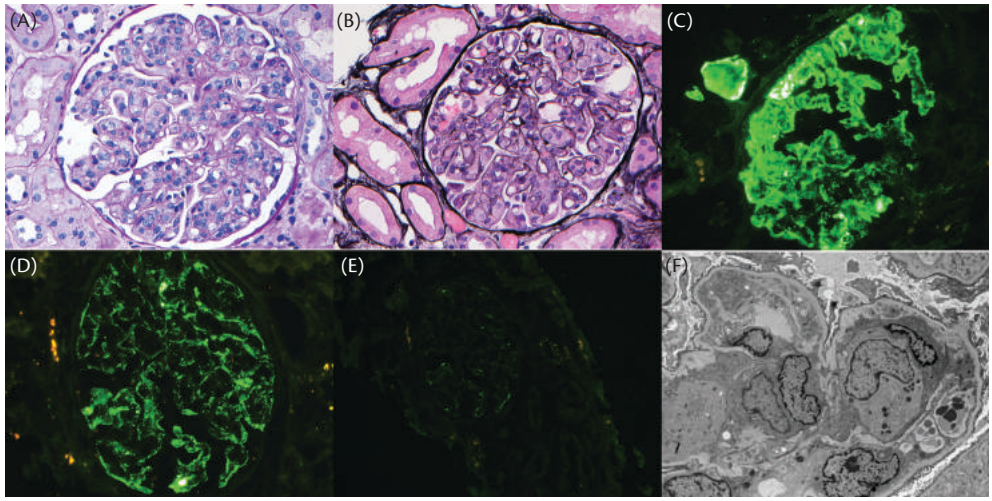

Fig. 155.1 Membranoproliferative glomerulonephritis. (A, B) Light microscopy showing proliferative changes with endocapillary proliferation with double contour formation (×40). (C, D, E) Immunofluorescence microscopy showing positive staining for (C) IgM and (D) kappa light chains and (E) negative lambda light chains (×40). (F) Electron microscopy showing thickening of the capillary walls with subendothelial deposits and new basement membrane formation resulting in double contours.

interposition and new basement membrane formation with double contours were also seen. The deposits were granular and substructures were typically absent. In four biopsies, scattered subepithelial deposits could be identified. The mesangium also contained electron dense deposits in 21 of the 28 cases. Podocytes showed segmental effacement of the foot processes and many of the capillary loops showed leucocyte infiltration. Tubuloreticular structures were absent in the endothelial cells.

Sixteen out of the 28 patients were classified as having a monoclonal gammopathy of undetermined significance (MGUS). The diagnosis of MGUS requires a serum monoclonal paraprotein band of < 30 g/L, a bone marrow biopsy that shows < 10% plasma cells, absence of lytic lesions, anaemia, and hypercalcaemia, and absence of end-organ damage. It is the most common plasma cell disorder recognized and is a potential precursor for MM (Kyle and Rajkumar, 2006; Kyle et al., 2006). In the remaining patients, MPGN was seen in the setting of other conditions associated with a monoclonal gammopathy, such as B-cell lymphomas, chronic lymphocytic leukaemia (CLL), and MM. In one case, serum electrophoresis studies were negative even though the renal biopsy suggested a MPGN secondary to monoclonal gammopathy. A few months after the biopsy, serum immunofixation results returned positive for a monoclonal gammopathy.

Others have also made similar observations. A proliferative glomerulonephritis mimicking ordinary immune complex-mediated glomerulonephritis associated with monoclonal IgG deposition and light-chain restriction has been described (Nasr et al., 2004, 2009). Nephrotic syndrome, renal insufficiency, and haematuria were present in 49%, 68%, and 77% of patients, respectively. Of 32 patients who were followed for over 2 years, 22% progressed to end-stage renal disease (ESRD), 38% had complete or partial recovery, and 38% had persistent renal dysfunction (Nasr et al., 2009). These studies, however, differ from our observations in that the deposits were composed exclusively of monoclonal IgG, and thus may represent a subgroup of the type of patients that we have described. Bone marrow biopsy was performed in 22 of 37 cases, of which only one patient showed MM. Because a monoclonal gammopathy was identified in only 30% of the cases, the authors did not associate the lesions with MGUS, lymphoproliferative disease, or MM. Recurrence of proliferative glomerulonephritis with monoclonal IgG deposits has been described in transplanted kidneys (Nasr et al., 2011). Finally, a few cases of a proliferative GN associated with a monoclonal IgA have also been reported (Akatsuka et al., 1997; Soares et al., 2006; Kaneko et al., 2010).

In summary, a significant proportion (40–50%) of idiopathic MPGN may be related to deposition of monoclonal intact immunoglobulins. Although a direct relationship between the presence of a monoclonal protein and the development of a MPGN remains to be proven, the present observations point in that direction. In approximately 60% of monoclonal protein-related MPGN, there is an associated asymptomatic MGUS. However, in the remaining cases, a more serious underlying lymphoid or plasma cell disorder can be detected and in these patients MPGN may often be the first sign of the underlying lymphoplasmacytic disorder. We recommend that in the assessment of idiopathic MPGN, renal biopsies be analysed with anti-light-chain antibodies to detect a possible underlying monoclonal gammopathy. Similarly, all patients with idiopathic MPGN should also undergo a full workup for monoclonal gammopathies, which should include serum and urine immunofixation studies. If positive, a bone marrow biopsy should also be performed. As it stands, 'idiopathic' MPGN appears to be a vanishing disease as a possible underlying aetiology is likely to be found in the majority of cases of MPGN.

Treatment

There is no standard treatment for patients with MPGN associated with a monoclonal gammopathy. Conservative as well as immunosuppressive therapy with the use of corticosteroids (alone or in combination with an alkylating agents), thalidomide, bortezomib (Velcade®), mycophenolate mofetil, ciclosporin, and rituximab have been used in a small number of patients with variable outcomes (Nasr et al., 2009; Guiard et al., 2011). Prospective, controlled

Table 155.1 Chemotherapy dosing

Regimen	Usual dosing schedule[a]
Bortezomib–dexamethasone	Bortezomib 1.3 mg/m^2 subcutaneously days 1, 8, 15, 22 Dexamethasone 40 mg days 1, 8, 15, 22 Repeated every 4 weeks
Bortezomib–cyclophosphamide–dexamethasone (VCD)	Cyclophosphamide 300 mg/m^2 orally on days 1, 8, 15 and 22 Bortezomib 1.3 mg/m^2 subcutaneously on days 1, 8, 15, 22 Dexamethasone 40 mg orally on days on days 1, 8, 15, 22 Repeated every 4 weeks; day 22 dosing can be omitted if blood counts are low or if patient experiences toxicity
Rituximab[b]	375 mg/m^2 in 0.9% saline infused intravenously on days 1, 8, 15, 22

[a] All doses need to be adjusted for performance status, blood counts, and other toxicities; cyclophosphamide requires dose reduction in presence of renal failure.

[b] Requires premedication, and adherence to infusion requirements per manufacturers prescribing guidelines.

studies in larger cohorts of patients with MPGN and monoclonal gammopathy are needed to ascertain optimal therapy. At the present time, treatment decisions will have to be made purely based on clinical experience.

In patients with an underlying malignancy such as myeloma, lymphoma, or CLL, therapy should be directed to controlling the malignancy and the monoclonal protein levels. In patients without an underlying malignancy but just MGUS, specific treatment for the associated MPGN if needed will differ based on the type of the immunoglobulin. In patients with non-IgM monoclonal proteins, a regimen of bortezomib plus dexamethasone (Vd) or bortezomib, cyclophosphamide, and dexamethasone (VCd) similar to that used in myeloma is reasonable since these regimens are well tolerated, safe for use in renal failure, with minimal dose adjustments needed (Table 155.1). We typically treat patients for approximately 4 months and then reassess. Some patients may also respond to a 3–6-month combined course of prednisone and cyclophosphamide with disappearance of the monoclonal gammopathy. In patients with IgM monoclonal proteins, one cycle of rituximab (four doses of 375 mg/m^2) with or without corticosteroids is a reasonable option for initial therapy.

Acquired Fanconi syndrome

Light-chain proximal tubulopathy with Fanconi syndrome (LCFS) is a rare complication of plasma cell dyscrasias (Maldonado et al., 1975; Herlitz et al., 2009). The mechanism of injury is thought to be related to the toxicity of the intracellular light chain (typically κ) crystalline deposits in proximal tubule. The propensity for light chains in LCFS to form crystals that precipitate within the cytoplasm appears to be determined by the light chain physical and chemical properties. Amino acid sequencing and mass spectrometry of the crystals have shown that almost all of the amino acid fragments come from the terminal variable (V) domain (VK1 subgroup) together with a low proportion of the entire κ chain (Aucouturier et al., 1993; Rocca et al., 1995; Deret et al., 1999; Bridoux et al., 2005). In contrast to other monoclonal light chains, which are degraded in the lysosomal compartment of proximal tubular cells, light chains in patients with LCFS are resistant to proteolysis and have a propensity to self-aggregate and to form intracellular crystals (Aucouturier et al., 1993; Leboulleux et al., 1995). The precise mechanism involved in cellular toxicity is unknown: neither crystallization nor resistance to proteolysis can fully explain the functional abnormalities of the proximal tubular cells (Decourt et al., 1999; Messiaen et al., 2000). Regardless, as a result of the impairment of proximal tubular functions, patients develop a Fanconi syndrome manifested as normoglycaemic glycosuria, aminoaciduria, hyperphosphaturia, hypophosphataemia, and proximal (type II) renal tubular acidosis, but incomplete forms of Fanconi syndrome (no aminoaciduria) has also been reported (Decourt et al., 2003).

Pathology and clinical considerations

Light microscopy typically reveals an acute or chronic tubulointerstitial nephropathy (Herlitz et al., 2009). Crystals appear pale on haematoxylin and eosin and periodic acid–Schiff stains (Fig. 155.2A). IF microscopy often shows either bright crystalline appearing kappa or lambda light chains within the tubular epithelial cells. Pronase-digested material from the paraffin block can also be used to detect the light chains in LCFS (Nasr et al., 2006) (Fig. 155.2B, C). EM shows electron-dense crystalline structures within the cytoplasm of proximal tubular epithelial cells (Fig. 155.2D–F). The main differential diagnosis on kidney biopsies include acute tubular injury due to other causes, light chain cast nephropathy (intratubular casts), inflammatory tubulointerstitial nephritis associated with light chains and protein reabsorption droplets with monotypic light staining (in the absence of intracellular crystals) (Cornell, 2011). Light-chain deposition disease may coexist with LCFS.

Clinical presentation is very heterogeneous and can vary from mild renal dysfunction, proteinuria, or osteomalacia (as a result of hypophosphataemia) to clinically overt acute renal failure, AL amyloidosis, or MM (Maldonado et al., 1975; Rao et al., 1987; Messiaen et al., 2000; Decourt et al., 2003; Bridoux et al., 2005; Kapur et al., 2007). LCFS has also been reported in patients with CLL and small lymphocytic lymphoma (Thorner et al., 1983; Rao et al., 1987). In adults, Fanconi syndrome is usually a consequence of drug toxicity (e.g. heavy metal poisoning) or secondary to urinary excretion of a monoclonal light chain, usually of κ isotype (Lee et al., 1972). Laboratory tests to rule out a monoclonal gammopathy as a cause of LCFS include serum and urine protein electrophoresis and immunofixation, serum free light-chain quantification, and bone marrow biopsy. In general, patients with LCFS have a slowly progressive course, but some patients will progress to develop MM or AL amyloidosis (Engle and Wallis, 1957; Maldonado et al., 1975; Ma et al., 2004). In a series of 32 patients diagnosed with LCFS, Ma et al. reported that at presentation most patients had a MGUS or smouldering MM, with a median creatinine level of 2.0 mg/dL (range 0.9–3.7 mg/dL) (Ma et al., 2004). A renal biopsy was performed in 17 patients, but crystal deposits in proximal tubule cells were present in only eight cases. In this heterogeneous group of patients, follow-up of 65 months (range 2–238 months) showed that five patients developed ESRD, and only one of 14 patients with MGUS transformed to MM.

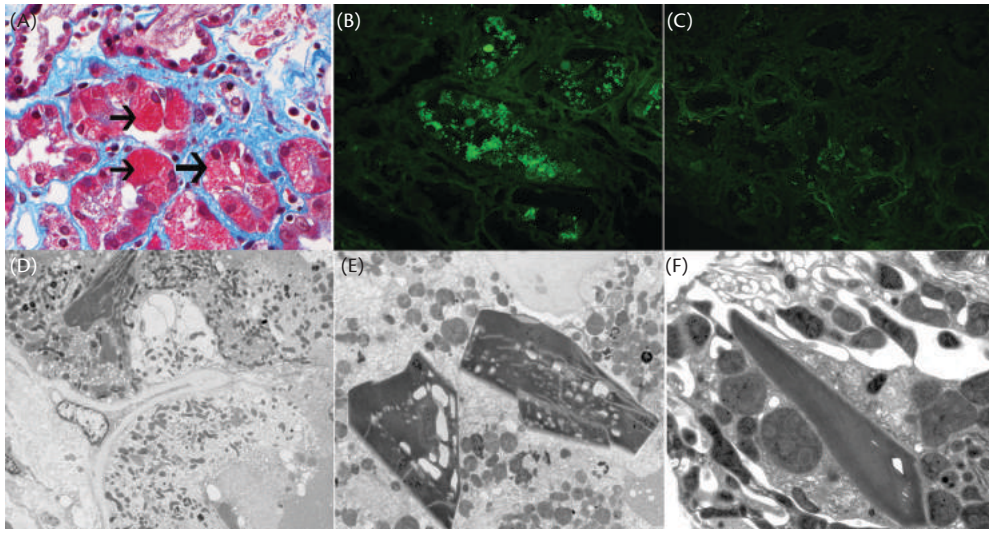

Fig. 155.2 Light chain tubulopathy (Fanconi syndrome). (A) Trichrome stain showing crystals within tubular epithelial cells, arrows point to crystals (×60). (B) Positive staining for kappa light chains. (C) Negative staining for lambda light chains. (D, E, F) Electron microscopy showing light chain crystals (D, ×4730; E, ×7830; F, ×17900).

Treatment

The optimal treatment of patients with LCFS remains unknown. In the study by Ma et al., all patients with MM or Waldenström macroglobulinaemia, four of six patients with smouldering MM, and six of 14 patients with MGUS were treated with chemotherapy (Ma et al., 2004). Fourteen patients died (one from ESRD, four from alkylator-related leukaemia or myelodysplastic syndrome). These observations suggest that chemotherapy offers little benefit on renal functions of patients with MGUS or smouldering MM, and it should be reserved for patients with evidence of overt malignancy or progressive lymphoproliferative disorder (Bridoux et al., 2005; Herlitz et al., 2009). The role of newer agents such as bortezomib and lenalidomide in the treatment of LCFS is not known.

As discussed earlier under treatment of MPGN, in the absence of data, treatment decisions currently are made primarily based on clinical opinion and experience. In patients with an underlying malignancy such as myeloma, lymphoma, or CLL, therapy should be directed to controlling the malignancy and the monoclonal protein levels. In patients without an underlying malignancy but just MGUS, a decision must first be made if the underlying LCFS is of a severity adequate to justify chemotherapy. If therapy is felt to be required, a strategy similar to the one outlined for MPGN associated with immunoglobulin deposition could be considered.

References

Akatsuka, T., Kawata, T., Hashimoto, S., *et al.* (1997). Rapidly progressive renal failure occurring in the course of pyoderma gangrenosum and IgA (lambda) monoclonal gammopathy. *Intern Med*, 36, 40–3.

Alpers, C. E., Tu, W. H., Hopper, J., Jr., *et al.* (1985). Single light chain subclass (kappa chain) immunoglobulin deposition in glomerulonephritis. *Hum Pathol*, 16, 294–304.

Aucouturier, P., Bauwens, M., Khamlichi, A. A., *et al.* (1993). Monoclonal Ig L chain and L chain V domain fragment crystallization in myeloma-associated Fanconi's syndrome. *J Immunol* 150, 3561–8.

Audard, V., Georges, B., Vanhille, P., *et al.* (2008). Renal lesions associated with IgM-secreting monoclonal proliferations: revisiting the disease spectrum. *Clin J Am Soc Nephrol*, 3, 1339–49.

Bridoux, F., Sirac, C., Hugue, V., *et al.* (2005). Fanconi's syndrome induced by a monoclonal Vkappa3 light chain in Waldenstrom's macroglobulinemia. *Am J Kidney Dis*, 45, 749–57.

Cornell, L. D. (2011). Light chain Fanconi syndrome. In R. B. Colvin (ed.) *Diagnostic Pathology Kidney Diseases*, pp. 245–7. Altona: Amirsys.

Decourt, C., Bridoux, F., Touchard, G., *et al.* (2003). A monoclonal V kappa l light chain responsible for incomplete proximal tubulopathy. *Am J Kidney Dis*, 41, 497–504.

Decourt, C., Rocca, A., Bridoux, F., *et al.* (1999). Mutational analysis in murine models for myeloma-associated Fanconi's syndrome or cast myeloma nephropathy. *Blood*, 94, 3559–66.

Deret, S., Denoroy, L., Lamarine, M., *et al.* (1999). Kappa light chain-associated Fanconi's syndrome: molecular analysis of monoclonal immunoglobulin light chains from patients with and without intracellular crystals. *Protein Eng*, 12, 363–9.

Dhar, S. K., Smith, E. C., and Fresco, R. (1977). Proliferative glomerulonephritis in monoclonal gammopathy. *Nephron*, 19, 288–94.

Engle, R. L., Jr. and Wallis, L. A. (1957). Multiple myeloma and the adult Fanconi syndrome. I. Report of a case with crystal-like deposits in the tumor cells and in the epithelial cells of the kidney. *Am J Med*, 22, 5–23.

Fakhouri, F., Darre, S., Droz, D., *et al.* (2002). Mesangial IgG glomerulonephritis: a distinct type of primary glomerulonephritis. *J Am Soc Nephrol*, 13, 379–87.

Guiard, E., Karras, A., Plaisier, E., *et al.* (2011). Patterns of noncryoglobulinemic glomerulonephritis with monoclonal Ig deposits: correlation with IgG subclass and response to rituximab. *Clin J Am Soc Nephrol*, 6, 1609–16.

Herlitz, L. C., Roglieri, J., Resta, R., *et al.* (2009). Light chain proximal tubulopathy. *Kidney Int*, 76, 792–7.

Kaneko, S., Usui, J., Narimatsu, Y., *et al.* (2010). Renal involvement of monoclonal immunoglobulin deposition disease associated with an unusual monoclonal immunoglobulin A glycan profile. *Clin Exp Nephrol*, 14, 389–95.

Kapur, U., Barton, K., Fresco, R., *et al.* (2007). Expanding the pathologic spectrum of immunoglobulin light chain proximal tubulopathy. *Arch Pathol Lab Med*, 131, 1368–72.

Kebler, R., Kithier, K., McDonald, F. D., *et al.* (1985). Rapidly progressive glomerulonephritis and monoclonal gammopathy. *Am J Med*, 78, 133–8.

Kyle, R. A. and Rajkumar, S. V. (2006). Monoclonal gammopathy of undetermined significance. *Br J Haematol*, 134, 573–89.

Kyle, R. A., Therneau, T. M., Rajkumar, S. V., *et al.* (2006). Prevalence of monoclonal gammopathy of undetermined significance. *N Engl J Med*, 354, 1362–9.

Leboulleux, M., Lelongt, B., Mougenot, B., *et al*. (1995). Protease resistance and binding of Ig light chains in myeloma-associated tubulopathies. *Kidney Int*, 48, 72–9.

Lee, D. B., Drinkard, J. P., Rosen, V. J., *et al*. (1972). The adult Fanconi syndrome: observations on etiology, morphology, renal function and mineral metabolism in three patients. *Medicine*, 51, 107–38.

Leung, N. and Rajkumar, S. V. (2007). Renal manifestations of plasma cell disorders. *Am J Kidney Dis*, 50, 155–65.

Ma, C. X., Lacy, M. Q., Rompala, J. F., *et al*. (2004). Acquired Fanconi syndrome is an indolent disorder in the absence of overt multiple myeloma. *Blood*, 104, 40–2.

Maldonado, J. E., Velosa, J. A., Kyle, R. A., *et al*. (1975). Fanconi syndrome in adults. A manifestation of a latent form of myeloma. *Am J Med*, 58, 354–64.

Masai, R., Wakui, H., Komatsuda, A., *et al*. (2009). Characteristics of proliferative glomerulo-nephritis with monoclonal IgG deposits associated with membranoproliferative features. *Clin Nephrol*, 72, 46–54.

Messiaen, T., Deret, S., Mougenot, B., *et al*. (2000). Adult Fanconi syndrome secondary to light chain gammopathy. Clinicopathologic heterogeneity and unusual features in 11 patients. *Medicine*, 79, 135–54.

Nasr, S. H., Galgano, S. J., Markowitz, G. S., *et al*. (2006). Immunofluorescence on pronase-digested paraffin sections: a valuable salvage technique for renal biopsies. *Kidney Int*, 70, 2148–51.

Nasr, S. H., Markowitz, G. S., Stokes, M. B., *et al*. (2004). Proliferative glomerulonephritis with monoclonal IgG deposits: a distinct entity mimicking immune-complex glomerulonephritis. *Kidney Int*, 65, 85–96.

Nasr, S. H., Satoskar, A., Markowitz, G. S., *et al*. (2009). Proliferative glomerulonephritis with monoclonal IgG deposits. *J Am Soc Nephrol*, 20, 2055–64.

Nasr, S. H., Sethi, S., Cornell, L. D., *et al*. (2011). Proliferative glomerulonephritis with monoclonal IgG deposits recurs in the allograft. *Clin J Am Soc Nephrol*, 6, 122–32.

Rao, D. S., Parfitt, A. M., Villanueva, A. R., *et al*. (1987). Hypophosphatemic osteomalacia and adult Fanconi syndrome due to light-chain nephropathy. Another form of oncogenous osteomalacia. *Am J Med*, 82, 333–8.

Rocca, A., Khamlichi, A. A., Touchard, G., *et al*. (1995). Sequences of V kappa L subgroup light chains in Fanconi's syndrome. Light chain V region gene usage restriction and peculiarities in myeloma-associated Fanconi's syndrome. *J Immunol*, 155, 3245–52.

Rosenstock, J. L., Markowitz, G. S., Valeri, A. M., *et al*. (2003). Fibrillary and immunotactoid glomerulonephritis: distinct entities with different clinical and pathologic features. *Kidney Int*, 63, 1450–61.

Sanders, P. W. (1993). Renal involvement in plasma cell dyscrasias. *Curr Opin Nephrol Hypertens*, 2, 246–52.

Sethi, S., Fervenza, F. C., Zhang, Y., *et al*. (2011). Proliferative glomerulonephritis secondary to dysfunction of the alternative pathway of complement. *Clin J Am Soc Nephrol*, 6, 1009–17.

Soares, S. M., Lager, D. J., Leung, N., *et al*. (2006). A proliferative glomerulonephritis secondary to a monoclonal IgA. *Am J Kidney Dis*, 47, 342–49.

Thorner, P. S., Bedard, Y. C., and Fernandes, B. J. (1983). Lambda-light-chain nephropathy with Fanconi's syndrome. *Arch Pathol Lab Med*, 107, 654–7.

Touchard, A. P. G., Hermine, O., and Ronco, P. (eds.) (2003). *Ultrastructural Pattern and Monoclonal Classification of Renal Monoclonal Immunoglobulin Deposits*. Dordrecht; Kluwer Academic Publisher.

Veltman, G. A., van Veen, S., Kluin-Nelemans, J. C., *et al*. (1997). Renal disease in Waldenstrom's macroglobulinaemia. *Nephrol Dial Transplant*, 12, 1256–9.

CHAPTER 156

The patient with sarcoidosis

Ravindra Rajakariar and Muhammad M. Yaqoob

Introduction

Sarcoidosis is a multisystem disorder with non-caseating granuloma being the pathological hallmark. It commonly affects the lymph nodes (typically bilateral hilar lymphadenopathy), lungs, skin, and the musculoskeletal system. It is a chronic relapsing–remitting disorder of unknown aetiology. Certain racial groups have a higher preponderance with Afro-Caribbean individuals having a 2.4% lifetime risk of developing sarcoidosis (Rybicki et al., 1997). Clinically significant renal involvement is uncommon and patients often present late in disease involvement.

Classification of renal disease in sarcoidosis

Renal tract involvement from sarcoidosis can be classified as follows:

- Impaired calcium homeostasis
- Granulomatous tubulointerstitial nephritis (TIN)
- Glomerular disease
- Obstructive uropathy.

Impaired calcium homeostasis

Disorders in calcium homeostasis leading to acute kidney injury (AKI) are the most common renal presentation in patients with sarcoidosis. Hypercalciuria occurs in > 50% of patients though hypercalcaemia is a presentation only in 10% (Sharma, 1996). The latter presentation is precipitated by exposure to sunlight. This could also occur due to prescription of high-dose cholecalciferol (D_3) or ergocalciferol (D_2) for native vitamin D deficiency. This is due to increased 1α-hydroxylase activity in granulomas that convert the native 25-hydroxyvitamin D_3 to the active 1,25-dihydroxyvitamin D_3, and therefore increased calcium reabsorption from gut and the resorptive effect of calcitriol on the bone (Mason et al., 1984; Insogna et al., 1988; Sharma, 1996). Hypercalciuria is often a visible feature with the presence of tubulointerstitial calcium oxalate crystals on native renal biopsies (Fig. 156.1A). This also can lead to symptomatic nephrolithiasis (Rizzato et al., 1995), polyuria, and chronic renal disease in the absence of granulomatous TIN (see following section).

Granulomatous tubulointerstitial nephritis

This is the second most common manifestation of sarcoidosis in the kidney. In itself it is an uncommon presentation but post-mortem studies have revealed a greater incidence of granulomatous TIN (Longcope and Freiman, 1952). It is commonly associated with systemic manifestations (Gobel et al., 2001), though in a small series of five patients (Robson et al., 2003) the sarcoid TIN was devoid of systemic involvement.

Patients often present with slowly progressive renal impairment with an insidious onset. As a consequence, it is detected late in the course of the disease process. The degree of renal impairment is therefore fairly advanced at the time of presentation. In a series of 17 patients with biopsy-proven TIN and sarcoidosis, the presenting mean Modification of Diet in Renal Disease (MDRD) 4-variable estimated glomerular filtration rate (eGFR) was 26.8 mL/min (Rajakariar et al., 2006). Proteinuria is usually present though < 1 g per 24 hours, typical of an interstitial renal disorder. Unless there is a dominant systemic involvement, serum angiotensin-converting enzyme levels are not raised. Histological appearances are in keeping with a non-caseating granulomatous TIN with a significant lymphocytic infiltrate in the interstitium associated with tubular atrophy and scarring (Fig. 156.1A–C). Intracellular calcification may be present in the absence of hypercalcaemia. There was no correlation with the degree of interstitial fibrosis and renal impairment (Rajakariar et al., 2006).

Glomerular disease

Secondary glomerular disease is an uncommon manifestation in sarcoidosis that presents with nephrotic syndrome. The commonest glomerular condition is membranous nephropathy. Other possible presentations include focal segmental glomerulosclerosis, minimal change disease, and immunoglobulin A disease (Rajakariar et al., 2006).

Obstructive uropathy

This is a rare complication of sarcoidosis and occurs due to bilateral ureteric obstruction secondary to retroperitoneal lymphadenopathy.

Pathogenesis

Aetiology for sarcoidosis has proven to be elusive. Infectious agents including mycobacteria, environmental agents (pesticide-using occupations, mould/mildew), occupational sources, and genetic susceptibility to the disease have been implicated. The mycobacterial tuberculosis catalase peroxidase (mKatG) has been identified as a potential antigen (Chen et al., 2008) and up to 50% of patients developed anti-mKatG antibodies compared to control subjects in one study (Song et al., 2005). Furthermore, following the World Trade Center attacks in 2001, a higher than anticipated incidence of pulmonary sarcoidosis was observed amongst New York City firefighters. The genetic associations include the butyrophilin-like

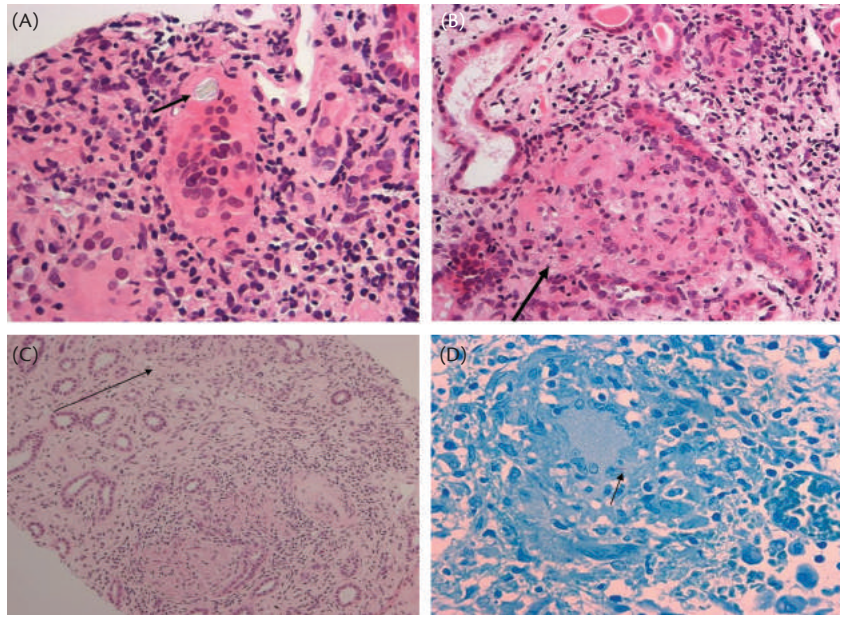

Fig. 156.1 Histological features of renal involvement in sarcoidosis. (A) Calcium oxalate crystals in lumen with features of adjacent granulomatous TIN. (B) Epithelioid macrophage rich granuloma (arrow) with associated lymphocytic infiltrate. (C) Marked interstitial scarring typical of chronic sarcoid TIN. (D) Acid-fast bacilli (arrow) when tissue stained with Ziehl–Neelsen in a patient with tuberculous TIN, which is otherwise indistinguishable from sarcoidosis.

protein 2 (*BTNL2*) and nucleotide-binding oligomerization domain containing 2 (*NOD2*) genes.

The key pathological feature is non-caseating granulomas and in its centre is a tightly knit group of macrophages, multinucleate giant cells, and epithelioid cells surrounded by lymphocytes rich with $CD4^+$ T lymphocytes relative to cytotoxic $CD8^+$ cells. In the circulation, however, the $CD4^+$ T cells are reduced compared to $CD8^+$ cells and are associated with a dominant T helper (Th)-1 cytokine response including increased tumour necrosis factor (TNF) alpha, interleukin 2, and gamma-interferon (IFN). These pro-inflammatory cytokines have a tissue-specific role in the pathogenesis of sarcoidosis and are responsible for development of granulomas and alveolar inflammation. The principal roles of granulomas are thought to be to confine the aetiological agent and to protect surrounding tissue by limiting the inflammatory damage.

Clinical presentation

In both hypercalcaemia and granulomatous TIN, patients present with AKI, mild to moderate proteinuria, and a bland urinary sediment with occasional leucocyturia. Hypercalciuria may be present. As mentioned previously, in granulomatous TIN, the onset of renal impairment could be more insidious with a stepwise decline in renal impairment. A patient may also present with AKI due to ureteric obstruction secondary to retroperitoneal lymph node involvement. Rarely, nephrolithiasis may present as renal colic.

Diagnosis

The diagnostic workup depends on the clinical presentation.

The initial investigations are blood for urea and electrolytes, bone profile, and C-reactive protein (CRP), urine for microscopy and assessment for proteinuria (protein creatinine ratio), together with a renal ultrasound scan will be sufficient in most cases. However, to diagnose TIN or a glomerular disease, the gold standard is a renal biopsy.

A renal biopsy is also indicated in the context of AKI ± hypercalcaemia as TIN can coexist with a raised serum calcium. Even though sarcoid granulomas produce angiotensin-converting enzyme, this is not often raised in TIN. If raised however, it could be a useful test to monitor disease activity. The Kveim–Stilbach test, which involves the intradermal injection of a homogenate of human sarcoid tissue, is not performed anymore due to lack of a commercial preparation and absence of validation.

Differential diagnosis

Granulomatous TIN in the context of sarcoidosis can be present in the absence of extrarenal manifestations. Often it remains a diagnosis of exclusion. The main differential is tuberculosis (TB)-induced granulomatous TIN (Chapagain et al., 2011). Sarcoidosis is common in certain ethnic groups such as South Asians in whom TB is commonly prevalent and often leads to a diagnostic dilemma. A positive Mantoux test or a TB gamma-IFN assay, presence of caseation in the granulomas, a positive Ziehl–Nielsen staining on biopsy specimens (Fig. 156.1D), and/or AFB culture favours the diagnosis of TB TIN. If sarcoidosis cannot be distinguished from TB, based on clinical presentation, demography, presence or absence of extrarenal involvement, and investigations, then treatment for both is recommended.

TIN with uveitis

This is a distinct condition with a female preponderance where patients present with flank pain, leucocyturia, microscopic haematuria with AKI, and with acute interstitial nephritis on the biopsy. Non-caseating granulomas can also occur. Uveitis is predominantly anterior. Both TIN with uveitis and Sjögren syndrome can present in a similar fashion and therefore other clinical clues are necessary to distinguish it from sarcoidosis.

Treatment

Corticosteroids, in spite of lack of evidence from high-quality randomized controlled trials, remain the first-line agent if treatment of sarcoidosis is indicated. For instance, in pulmonary sarcoidosis, corticosteroids are indicated in the event of either symptoms or stage 2–4 lung disease. In renal sarcoidosis, corticosteroids are indicated with AKI secondary to hypercalcaemia and biopsy-proven granulomatous TIN.

The starting dose is 0.5 mg/kg, doubled if the patient is also commenced on an empirical anti-tuberculous regimen that includes rifampicin (see 'Differential diagnosis'). Once disease control is achieved prednisolone can be tapered by 2.5 mg per week.

The steroid can be discontinued following a successful taper if the patient first presented with:

- AKI secondary to hypercalcaemia
- Granulomatous TIN with *no* evidence of chronic damage on an adequate renal biopsy sample.

However, as mentioned earlier, the majority of patients with granulomatous TIN present with advanced kidney injury with significant tubulointerstitial scarring (MDRD 4-variable eGFR < 60 mL/min) and therefore should be maintained on low dose of steroids (prednisolone 7.5 or 5 mg) (Rajakariar et al., 2006).

Alternatives to corticosteroids include methotrexate, mycophenolate mofetil (MMF), azathioprine, leflunomide, and anti-TNF therapies that include etanercept, infliximab, and adalimumab. The evidence base for the above steroid-sparing drugs is primarily from patients treated for pulmonary sarcoidosis. Steroid-sparing agents offer the advantage of decreased weight gain and hyperglycaemia, but with a greater prevalence of gastrointestinal side effects, leucopenia, infections, and raised liver transaminases.

Recurrence of sarcoidosis following transplantation

Patients with granulomatous TIN that reach CKD stage 5 will be considered for transplantation. As transplant recipients are on immunosuppression including MMF (or azathioprine) and prednisolone, recurrent disease is rare. In the event this was to occur, third-line agents such as anti-TNF therapies need consideration.

References

Chapagain, A., Dobbie, H., Sheaff, M., *et al.* (2011). Presentation, diagnosis, and treatment outcome of tuberculous-mediated tubulointerstitial nephritis. *Kidney Int*, 79(6), 671–7.

Chen, E. S., Wahlstrom, J., Song, Z., *et al.* (2008). T cell responses to mycobacterial catalase-peroxidase profile a pathogenic antigen in systemic sarcoidosis. *J Immunol*, 181(12), 8784–96.

Gobel, U., Kettritz, R., Schneider, W., *et al.* (2001). The protean face of renal sarcoidosis. *J Am Soc Nephrol*, 12(3), 616–23.

Insogna, K. L., Dreyer, B. E., Mitnick, M., *et al.* (1988). Enhanced production rate of 1,25-dihydroxyvitamin D in sarcoidosis. *J Clin Endocrinol Metab*, 66(1), 72–5.

Longcope, W. T. and Freiman, D. G. (1952). A study of sarcoidosis; based on a combined investigation of 160 cases including 30 autopsies from The Johns Hopkins Hospital and Massachusetts General Hospital. *Medicine (Baltimore)*, 31(1), 1–132.

Mason, R. S., Frankel, T., Chan, Y. L., *et al.* (1984). Vitamin D conversion by sarcoid lymph node homogenate. *Ann Intern Med*, 100(1), 59–61.

Rajakariar, R., Sharples, E. J., Raftery, M. J., *et al.* (2006). Sarcoid tubulo-interstitial nephritis: long-term outcome and response to corticosteroid therapy. *Kidney Int*, 70(1), 165–9.

Rizzato, G., Fraioli, P., and Montemurro, L. (1995). Nephrolithiasis as a presenting feature of chronic sarcoidosis. *Thorax*, 50(5), 555–9.

Robson, M. G., Banerjee, D., Hopster, D., *et al.* (2003). Seven cases of granulomatous interstitial nephritis in the absence of extrarenal sarcoid. *Nephrol Dial Transplant*, 18(2), 280–4.

Rybicki, B. A., Major, M., Popovich, J. Jr., *et al.* (1997). Racial differences in sarcoidosis incidence: a 5-year study in a health maintenance organization. *Am J Epidemiol*, 145(3), 234–1.

Sharma, O. P. (1996). Vitamin D, calcium, and sarcoidosis. *Chest*, 109(2), 535–9.

Song, Z., Marzilli, L., Greenlee, B. M., *et al.* (2005). Mycobacterial catalase-peroxidase is a tissue antigen and target of the adaptive immune response in systemic sarcoidosis. *J Exp Med*, 201(5), 755–67.

CHAPTER 157

The patient with vasculitis: overview

David Jayne

Introduction

The term vasculitis indicates a histological triad of blood vessel wall inflammation, fibrinoid necrosis, and thrombotic occlusion. This process can occur in any tissue in the body and results from a variety of immunologic, infective, or other causes. Vasculitis syndromes refer to a group of diseases where vasculitis is the predominant histological feature and should be differentiated from non-vasculitic causes of vascular disease. These syndromes may occur as primary, presumed autoimmune syndromes, or are secondary to other disease processes. Patients with vasculitis present to a wide range of different medical specialties depending on the organ involvement and the varied presentation, complexities of classification, and diagnosis have impeded early diagnosis and commencement of therapy.

Vasculitis is the most common cause of rapidly progressive glomerulonephritis and is the cause of end-stage renal failure in 3–4% of all cases. Unlike many other forms of renal disease, vasculitis is treatable and renal progression preventable provided there is prompt diagnosis and treatment. Thus, suspicion of a vasculitis syndrome is a key step and usually one not performed by the nephrologist. The current understanding of the classification of vasculitis syndromes is described along with descriptions of clinical presentations, and approaches to diagnosis.

Vasculitis classification

History

Classification has developed from descriptions of individual cases, as in Henoch–Schönlein purpura and polyarteritis nodosa in the 1800s, to small series, Wegener's granulomatosis in 1936, and Churg–Strauss syndrome in 1951 (Churg and Strauss, 1951; Fahey et al., 1954). Often based on post-mortem studies, the descriptions linked a pattern of clinical features with histological characteristics. Grouping of vasculitic syndromes according to the size of blood vessel involved was proposed by Pearl Zeek in 1948 and a data-driven attempt to develop classification criteria reported in 1990 by the American College of Rheumatology (ACR) (Zeek et al., 1948; Hunder et al., 1990). This system did not include antineutrophil cytoplasmic antibodies (ANCAs), discovered in the early 1980s, and was limited by differences in terminology and the exclusion of microscopic polyangiitis (MPA). In 1993, the International Chapel Hill Consensus Conference (CHCC) agreed a set of definitions for the major subgroups of primary systemic vasculitis (Jennette et al., 1994). This recognized that the renal histology of Wegener's granulomatosis and MPA was similar and featured few or no immune deposits, 'pauci-immune', and that a renal-limited form of pauci-immune vasculitis existed. Previously, nephrologists had recognized MPA when a pauci-immune necrotizing glomerulonephritis was associated with extrarenal vasculitis, but had used the term 'idiopathic rapidly progressive, or crescentic, glomerulonephritis' to describe renal-limited presentations.

An update of the 1993 CHCC definitions was published in 2012 that also replaced some of the eponyms with descriptive terms (Fig. 157.1, Table 157.1) (Jennette et al., 2013). An initial distinction was made between primary, no identifiable cause, and secondary vasculitis; the small vessel group was subdivided into those syndromes associated with ANCA and those with immune complex deposition on tissue biopsy, and a new category, single-organ vasculitis, was included.

Classification and diagnostic criteria

Although there are no agreed diagnostic criteria for vasculitis syndromes, adaptation of the CHCC definitions or ACR classification criteria have been employed successfully in clinical trials (Table 157.2) (Jayne et al., 2003; Wegener's Granulomatosis Etanercept Trial Research Group, 2005). Studies by the European Vasculitis Study Group in ANCA-associated vasculitis have required a compatible clinical presentation with either a positive ANCA or confirmatory tissue biopsy, and the exclusion of other causes for the presentation (Table 157.2). The presence of proteinase 3 (PR3)-ANCA has been added as a fifth item to the ACR classification criteria for Wegener's granulomatosis with polyangiitis (Wegener's) (GPA) in North American studies. Long-term follow-up has indicated a high specificity for these criteria but the sensitivity is not known. Classification criteria for paediatric vasculitis have been developed from expert opinion and a data-driven study (Ozen et al., 2009).

The major overlaps in clinical phenotype and histology between ANCA vasculitis syndromes complicate classification of individual patients. Granulomatous inflammation is required for GPA but is often hard to demonstrate on biopsy, and although eosinophil infiltration is a defining feature of eosinophilic GPA (EGPA), it can also be found in GPA biopsies. Differences in genetic susceptibility between PR3-ANCA and myeloperoxidase (MPO)-ANCA vasculitis patients, and the stronger associations of baseline phenotype and outcomes with serology (PR3 or MPO-ANCA) than phenotype (GPA or MPA) is driving re-assessment of the current classification of ANCA vasculitis (Lyons et al., 2012).

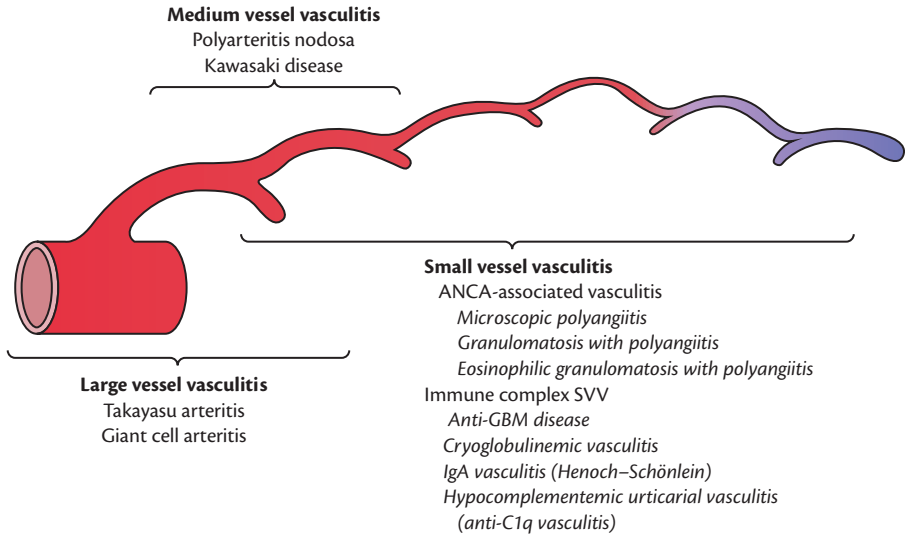

Fig. 157.1 Terminology of the major primary vasculitis syndromes classified according to the predominant size of blood vessel involved (Chapel Hill Consensus Conference, 2012).
From Jennette et al. (2013).

Table 157.1 Definitions of the major primary vasculitis syndrome classified according to the predominant size of blood vessel involved

CHCC 2012 names	CHCC 2012 definitions
Large vessel vasculitis (LVV)	Vasculitis affecting large arteries more often than other vasculitides. Large arteries are the aorta and its major branches. Any size artery may be affected
Takayasu arteritis (TAK)	Arteritis, often granulomatous, predominantly affecting the aorta and/or its major branches. Onset usually in patients < 50 years old
Giant cell arteritis (GCA)	Arteritis, often granulomatous, usually affecting the aorta and/or its major branches, with a predilection for the branches of the carotid and vertebral arteries. Often involves the temporal artery. Onset usually in patients > 50 years old and often associated with polymyalgia rheumatica
Medium vessel vasculitis (MVV)	Vasculitis predominantly affecting medium arteries defined as the main visceral arteries and their branches. Any size artery may be affected. Inflammatory aneurysms and stenoses are common
Polyarteritis nodosa	Necrotizing arteritis of medium or small arteries without glomerulonephritis or vasculitis in arterioles, capillaries, or venules; and not associated with ANCA
Kawasaki disease	Arteritis associated with the mucocutaneous lymph node syndrome and predominantly affecting medium and small arteries. Coronary arteries are often involved. Aorta and large arteries may be involved. Usually occurs in infants and young children
Small vessel vasculitis (SVV)	Vasculitis predominantly affecting small vessels, defined as small intraparenchymal arteries, arterioles, capillaries and venules. Medium arteries and veins may be affected
ANCA-associated vasculitis (AAV)	Necrotizing vasculitis, with few or no immune deposits, predominantly affecting small vessels (i.e. capillaries, venules, arterioles and small arteries), associated with MPO-ANCA or PR3-ANCA. Not all patients have ANCA. Add a prefix indicating ANCA reactivity (e.g. PR3-ANCA, MPO-ANCA, ANCA-negative)
Microscopic polyangiitis (MPA)	Necrotizing vasculitis, with few or no immune deposits, predominantly affecting small vessels (i.e. capillaries, venules, or arterioles). Necrotizing arteritis involving small and medium arteries may be present. Necrotizing glomerulonephritis is very common. Pulmonary capillaritis often occurs. Granulomatous inflammation is absent
Granulomatosis with polyangiitis (Wegener's) (GPA)	Necrotizing granulomatous inflammation usually involving the upper and lower respiratory tract, and necrotizing vasculitis affecting predominantly small to vessels (e.g. capillaries, venules, arterioles, arteries and veins). Necrotizing glomerulonephritis is common
Eosinophilic granulomatosis with polyangiitis (Churg–Strauss) (EGPA)	Eosinophil-rich and necrotizing granulomatous inflammation often involving the respiratory tract, and necrotizing vasculitis predominantly affecting small to medium vessels, and associated with asthma and eosinophilia. ANCA is more frequent when glomerulonephritis is present

(*Continued*)

Table 157.1 Continued

CHCC 2012 names	CHCC 2012 definitions
Immune complex vasculitis	Vasculitis with moderate to marked vessel wall deposits of immunoglobulin and/or complement components predominantly affecting small vessels (i.e. capillaries, venules, arterioles and small arteries). Glomerulonephritis is frequent
Anti-GBM disease	Vasculitis affecting glomerular capillaries, pulmonary capillaries, or both, with basement membrane deposition of anti-basement membrane autoantibodies. Lung involvement causes pulmonary haemorrhage, and renal involvement causes glomerulonephritis with necrosis and crescents
Cryoglobulinaemic vasculitis	Vasculitis with cryoglobulin immune deposits affecting small vessels (predominantly capillaries, venules, or arterioles) and associated with cryoglobulins in serum. Skin, glomeruli, and peripheral nerves are often involved
IgA vasculitis (Henoch–Schönlein)	Vasculitis, with IgA1-dominant immune deposits, affecting small vessels (predominantly capillaries, venules, or arterioles). Often involves skin and gut, and frequently causes arthritis. Glomerulonephritis indistinguishable from IgA nephropathy may occur
Hypocomplementaemic Urticarial vasculitis (HUV) (anti-C1q vasculitis)	Vasculitis accompanied by urticaria and hypocomplementaemia affecting small vessels (i.e. capillaries, venules, or arterioles), and associated with anti-C1q antibodies. Glomerulonephritis, arthritis, obstructive pulmonary disease, and ocular inflammation are common

CHCC 2012 = Chapel Hill Consensus Conference, 2012.

From Jennette et al. (2013).

Attempts have been made to find subgroups within EGPA based on organ involvement and the presence of ANCA. Although there are trends for nephritis and neuropathy to be associated with a positive ANCA and for cardiac and gastroenterological disease to be associated with a negative ANCA and higher eosinophil count, the distinctions are not strong enough for a clinically useful system (Sable-Fourtassou et al., 2005; Sinico et al., 2005).

Epidemiology

ANCA-associated vasculitis

Population-based studies have indicated a prevalence of 250 per million, higher than that estimated by clinic-based surveys (50–10 per million) (Mohammad et al., 2007). With improved case ascertainment and survival this figure is likely to increase.

Table 157.2 Eligibility criteria for clinical trials of ANCA-associated vasculitis

European Vasculitis Study Group (all three required)	Amended American College of Rheumatology 1990 criteria for GPA (Wegener's) (at least two required)
1. Compatible clinical presentation for GPA, MPA, or renal-limited vasculitis	1. Nasal or oral inflammation (oral ulceration or purulent or bloody nasal discharge)
2. ANCA positive (C-ANCA or PR3-ANCA or MPO-ANCA) *and/or* biopsy confirmation of vasculitis	2. Abnormal chest radiograph (nodules, fixed infiltrates, or cavities)
3. Exclusion of other causes for the presentation	3. Microscopic haematuria (> 5 red cells per high powered field) or red cell casts
	4. Granulomatous inflammation on biopsy (within the arterial wall or in the perivascular or extravascular area)
	5. Positive PR3-ANCA

Jayne et al., 2003; Wegener's Granulomatosis Etanercept Trial Research Group, 2005

The incidence has been stable in a UK cohort over 25 years of observation at 19 per million per year. Of these, 70% have renal involvement and 30–50% acute kidney injury at presentation. The incidence figure is the same in Japan but the pattern of syndrome differs (Table 157.3) (Fujimoto et al., 2011). The frequency is lower in black and Asian populations in Europe (Mahr et al., 2004). A latitudinal gradient exists in both northern and southern hemispheres with GPA and EGPA being more frequent in colder, temperate climates and MPA more frequent in warmer climates (Gatenby et al., 2009).

The first genome-wide association study in GPA/MPA found genetic associations with GPA or PR3-ANCA but not MPA or MPA-ANCA, indicating that the aetiology of these syndromes was fundamentally different (Lyons et al., 2012). Associations were found with major histocompatibility complex (MHC) class II, *SERPINA1* (alpha-1 anti-trypsin) and *PRTN3* (proteinase 3). Previously reported candidate gene associations with *PTPN22* and *CTLA4* were not seen but this may reflect insensitivity of the genomic study (Martorana et al., 2012). Candidate gene associations of MHC and interleukin-10 (*IL-10*) with MPA and EGPA await confirmation.

The peak age of onset for GPA is 50–70 years but it can present in childhood; there is a female sex bias in younger patients. MPA has an older age distribution, peak age 60–80 years. The frequent respiratory tract involvement in GPA has indicated a causal role for environmental agents and Wegener himself suspected tuberculosis without direct bacteriological evidence. Nasal colonization

Table 157.3 Incidence (cases per million population per year) of ANCA vasculitis and GPA (Wegener's), MPA, and EGPA (Churg–Strauss) in Europe and Japan

	Total AAV	GPA (Wegener's)	MPA	EGPA (Churg–Strauss)
Europe	21.8	14.3	6.5	0.9
Japan	22.6	2.1	18.2	2.4

Fujimoto et al., (2011).

with *Staphylococcus aureus* has been associated with disease flares and ANCA production but it is unclear whether this is specific to this organism, and other infections also contribute to persisting or relapsing disease (Tadema et al., 2011). Molecular mimicry between microbial antigens and ANCA has been suggested by identification of anti-leucocyte associated membrane protein 2 (LAMP2) antibodies in vasculitis patients (Kain et al., 2012).

Occupational exposure to silica has a dose-dependent relationship with MPO-ANCA and MPA, and drugs, such as hydralazine and penicillamine, can induce an ANCA-associated vasculitis (AAV) often with several autoantibody reactivities (Hogan et al., 2007). Cocaine and amphetamines can cause a non-inflammatory vasculopathy but cocaine has also been associated with an upper respiratory tract disease similar to GPA in which anti-elastase antibodies are found. Recently, contamination of cocaine with levamisole has been linked with a systemic AAV with nephritis (Graf, 2013).

Immunoglobulin A vasculitis (Henoch–Schönlein purpura)

Immunoglobulin A (IgA) vasculitis is the most common vasculitis found in children with an incidence of 150–200 per million children per year, and is more frequent in Asian and less frequent in black children (Gardner-Medwin et al., 2002). The incidence in adults is 3–14 per million per year (Watts et al., 1998). Candidate gene studies have indicated potential associations of aetiology or severity with polymorphisms in complement genes, *CTLA4*, and angiotensin-converting enzyme (Yoshioka et al., 1998). Upper respiratory tract infection with bacteria and viruses are triggers of IgA vasculitis. Adults with IgA vasculitis have a fivefold relative risk for a concurrent diagnosis of malignancy compared with the general population (Pankhurst et al., 2004).

Cryoglobulinaemic vasculitis

Renal involvement is frequent in 'mixed' type II cryoglobulinaemia. This arises most commonly in the context of hepatitis C virus infection and an immune susceptibility associated with human leucocyte antigen DR11 has been suggested (Cacoub et al., 2001). Type II cryoglobulinaemia may be associated with other infections, such as parvovirus, with Sjögren syndrome, or with non-Hodgkin lymphoma (Matignon et al., 2009).

Presenting features

The early diagnosis of a vasculitic syndrome reduces the risk of end-stage renal failure and death. Diagnostic delays have been improving, but this remains a most important aspect of management over which the nephrologist has relatively little control (Takala et al., 2008). Patients with extrarenal disease present earlier with associated symptoms and, especially for those with ear, nose, and throat (ENT) disease, this translates into improved outcomes (de Lind van Wijngaarden et al., 2006). Those with renal-limited disease are at risk of late presentation with a high creatinine and symptoms of acute kidney injury.

Prodrome

ANCA-associated vasculitis

The majority of patients have a history of prodromal symptoms that precedes their diagnosis by an average of 6 months. These include polymyalgia, polyarthralgia, headache, malaise, fevers, night sweats, flitting arthritis, and weight loss. This is associated with elevated C-reactive protein and erythrocyte sedimentation rate, as well as urinary abnormalities. It is important that patients with such non-specific symptomatology are further investigated and the possibility of an autoimmune aetiology considered.

IgA vasculitis

Typically an upper respiratory tract infection precedes the emergence of a purpuric rash starting in the legs then spreading to the buttocks and trunk. Forty-eight per cent develop evidence of nephritis, 2–4 weeks after the development of a purpuric rash. Nephritis occurs in 48% and is more frequent with older age of onset (> 8 years), abdominal symptoms, and recurrent disease. It can present as isolated or combined haematuria or proteinuria, nephrotic syndrome, or rapidly progressive glomerulonephritis (Jauhola et al., 2010).

Features of renal disease

The frequency of glomerulonephritis in AAV syndromes is 70% in GPA, 90% in MPA, and 15% in EGPA (Sable-Fourtassou et al., 2005; Corral-Gudino et al., 2011). Urinary abnormalities are almost universal in small vessel vasculitis where the focus of renal injury is the glomerulus. They are characterized by microscopic or macroscopic haematuria, which can appear as brown smoky urine, with proteinuria. In ANCA vasculitis, nephrotic range proteinuria is rare as a presenting feature but this is more common in immune complex vasculitis. The presence of red cell casts in a freshly voided specimen is strongly indicative of a crescentic glomerulonephritis. Infection and other causes of lower urinary tract bleeding should be excluded. As the renal disease advances then fluid retention, hypertension, an elevated serum creatinine, and ultimately uraemic symptoms will appear. Renal imaging is either normal or demonstrates enhanced cortico-medullary differentiation, with normal arterial and venous blood flows. Rare renal features of ANCA vasculitis include renal rupture with pain and retro-peritoneal haemorrhage, and ureteric vasculitis with hydronephrosis in EGPA. Other causes or haematuria in vasculitis patients include prostatic vasculitis in GPA and cystitis associated with cyclophosphamide.

Polyarteritis nodosa is now a rare diagnosis because current classification moves patients with both medium and small vessel vasculitis into the MPA category. Renal involvement in polyarteritis nodosa is manifested by hypertension, wedge-shaped renal infarction on renal imaging, and only minor or no urinary abnormalities. Renal angiography may demonstrate aneurysm formation but the lack of angiographic abnormalities does not exclude the diagnosis (Fig. 157.2).

Renal artery disease is seen in 25% of patients with large vessel vasculitis and is usually asymptomatic with no urinary features and is often detected when investigating a patient with hypertension or with vascular disease in other territories (Grayson et al., 2012). It may be unilateral or bilateral and the pattern of renal artery stenosis differs from atheromatous disease with long, smooth, thick-walled stenoses commencing at the origin of the vessel from the aorta. The associated kidney may be small with reduced function on isotope imaging. Renal artery stenosis is also seen in the antiphospholipid syndrome.

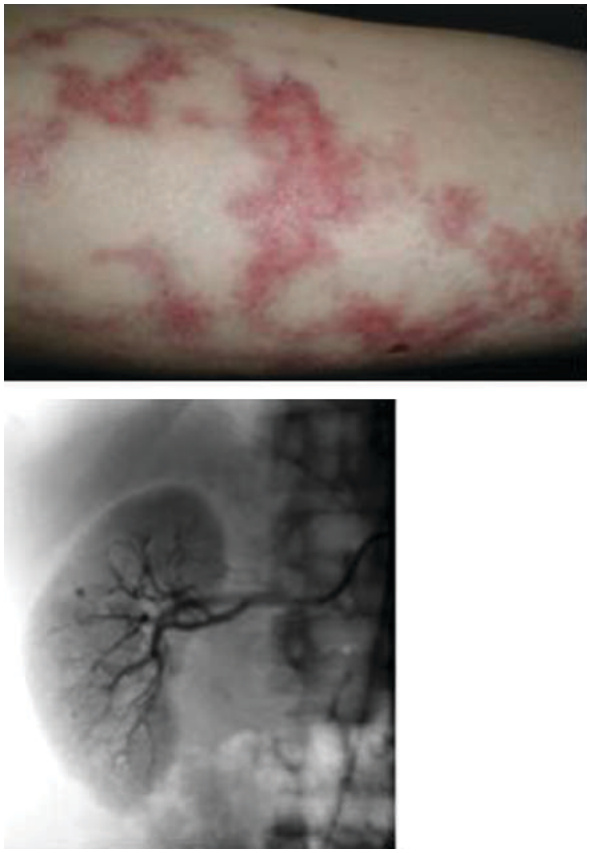

Fig. 157.2 Polyarteritis nodosa. Skin appearance and renal aneurysm.

Features of extrarenal disease

ANCA-associated vasculitis

GPA is characterized by granulomatous inflammation of the respiratory tract that presents as sinusitis, nasal crusting and epistaxis, collapse of the nasal bridge, deafness, trachea-bronchial stenoses, and cavitating lung disease. Less common granulomatous features are retro-orbital granuloma, perforating scleritis, pachymeningitis, and pituitary disease. Vasculitic features of rash, mononeuritis multiplex, alveolar infiltrates with or without cough or haemoptysis, episcleritis and scleritis, and gastrointestinal vasculitis are seen in both GPA and MPA. Cardiac disease is seen in 10% of GPA patients typically involving the aortic valve, but > 50% of EGPA patients have evidence of myocarditis.

The pulmonary-renal syndrome refers to the co-occurrence of diffuse alveolar haemorrhage and rapidly progressive glomerulonephritis. ANCA vasculitis is the most frequent cause, either in the setting of GPA or MPA, and less common causes are antiglomerular basement membrane (anti-GBM) disease, systemic lupus erythematosus (SLE), IgA vasculitis, or cryoglobulinaemic vasculitis (Hruskova et al., 2013). Severe alveolar haemorrhage without nephritis is very uncommon in AAV. Early recognition of this presentation is of particular importance because alveolar haemorrhage causing respiratory failure is the major vasculitic cause of early death (Flossmann et al., 2011).

Although the vasculitis manifestations seen in GPA/MPA also occur in EGPA, the latter is distinguished by asthma, naso-sinus disease with non-haemorrhagic rhinitis and nasal polyps, eosinophilic cardiomyopathy, and gastrointestinal disease.

Approaches to diagnosis

Suspicion of vasculitis

Because the presenting features of vasculitis are so variable and often non-specific if vasculitis is not suspected, diagnosis will be delayed. A vasculitic syndrome should be suspected in patients presenting with constitutional disturbance, which is unexplained and persistent, when there is focal extrarenal disease which could be part of a vasculitis syndrome, when there are unexplained renal features of disease, or in the setting of another disease process where a secondary vasculitis is common (Fig. 157.3).

Investigation for suspected vasculitis

Further investigation of a patient with suspected vasculitis aims firstly, to identify a pattern of presenting features that makes a vasculitis diagnosis possible by integrating the clinical features with serology, histology, and radiology. Secondly, to define the extent and severity of the disease, with respect to organ distribution and function; and thirdly to identify either secondary causes of the vasculitis syndrome or other diseases that might mimic a vasculitis presentation (Fig. 157.3). The Birmingham Vasculitis Activity Score is a 63-item tool divided into nine organ sections. Although developed for clinical trials it can serve as a checklist for the investigation of a patient with suspected vasculitis.

ANCA testing should include both indirect immunofluorescence (IIF) and solid phase assays for PR3 and MPO-ANCA. There is no standardization of ANCA assays and there is large variance in performance (Holle et al., 2012). IIF is usually more sensitive, but less specific, than antigen-specific assays. A positive antinuclear antibody (ANA) can produce a false-positive perinuclear (P)-ANCA pattern. In a patient with suspected nephritis, positivity for cytoplasmic (C)-ANCA and PR3-ANCA or P-ANCA and MPO-ANCA has a 95% positive predictive value for a biopsy demonstrating renal vasculitis. ANCA is negative in 10–15% of biopsy-proven, pauci-immune glomerulonephritis so a negative ANCA does not exclude the diagnosis. Complement levels are normal in AAV and low levels in conjunction with a positive rheumatoid factor and monoclonal band on serum protein electrophoresis suggests cryoglobulinaemia.

For small vessel vasculitis syndromes, the investigation pathway should include tissue biopsy with immunohistology, where possible, because this increases diagnostic certainty and subgroup classification, and renal histology has prognostic significance (Table 157.1) (Berden et al., 2010). Limited non-renal presentations of AAV may be ANCA negative and respiratory tract histology has a relatively low diagnostic yield. Specialist ENT and respiratory opinion combined with response to therapy and a period of observation will increase or reduce confidence in the diagnosis. Polyarteritis nodosa without vital organ involvement is also problematic because specific serological markers are absent and aneurysms may not be present in early or non-severe cases. The cutaneous appearance with arteritis on a deep skin biopsy or muscle biopsy may confirm the diagnosis. Histology is available in a minority of patients with large vessel vasculitis with renal artery involvement if there is a classic giant cell arteritis presentation and temporal artery biopsy or surgical reconstruction, such as aortic root replacement. In the majority,

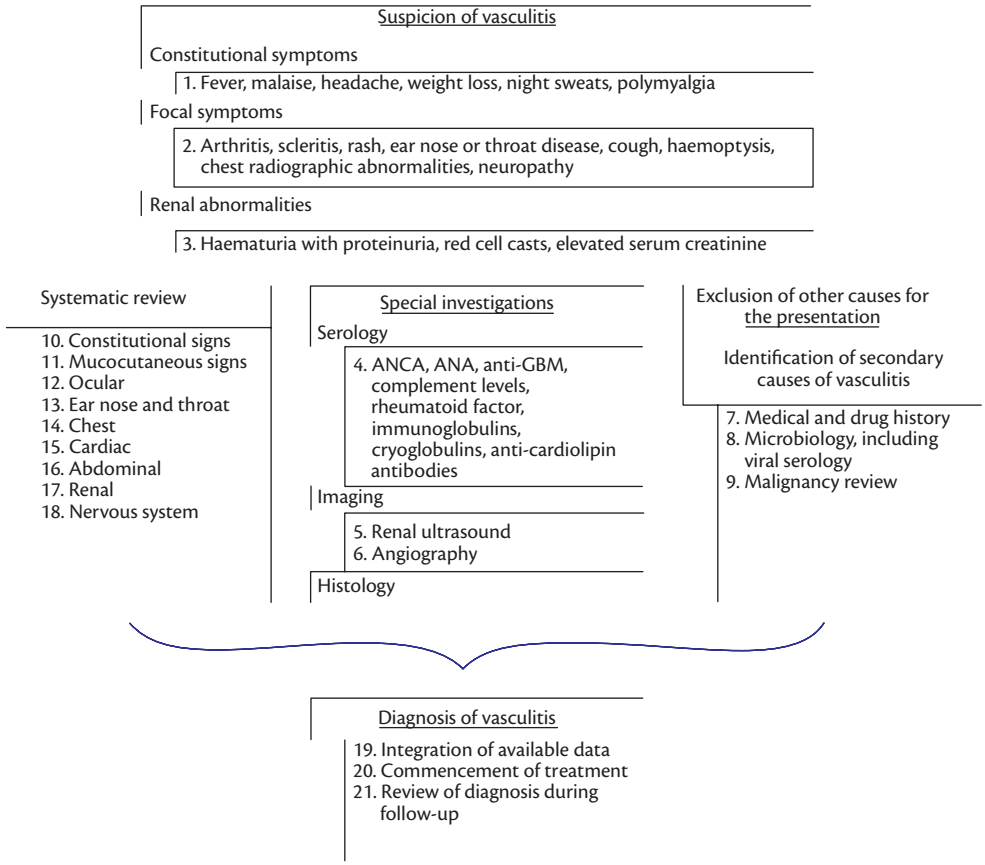

Fig. 157.3 An approach to diagnosis in a patient with suspected vasculitis.

diagnosis relies on the angiographic pattern of stenosis or aneurysms with vascular inflammation, supported by presenting features, elevated inflammatory marker and a positive FDG-positron emission tomography scan.

Diagnosis of vasculitis

There are no established diagnostic criteria to differentiate vasculitis from other diseases. Once vasculitis is thought probable, allocation to a vasculitic syndrome has been standardized in small and medium vessel vasculitides by an algorithm developed for the European Medical Evaluation Agency (Fig. 157.4) (Watts et al., 2007). The1990 ACR classification criteria with subsequent modifications function reasonably well for large vessel vasculitis (Basu et al., 2010).

Secondary causes of vasculitis

Chronic infections with hepatitis C can cause a direct arteritis or through dysregulated B-cell activity, a cryoglobulinaemic vasculitis. Hepatitis B has been associated with polyarteritis nodosa but this has become very rare in the Western world. Human immunodeficiency virus causes inflammatory syndromes including vasculitis (Patel et al., 2011). Chronic bacterial infection, especially in the setting of immunodeficiency, cystic fibrosis, bronchiectasis, and infective endocarditis, increases the risk of both an immune complex vasculitis and an MPO-ANCA-positive MPA. Tuberculosis is an important mimic of vasculitis but ANCA vasculitis may coexist with active or latent tuberculosis (Gregersen et al., 2013).

Malignancy is present at the time of diagnosis of 1–4% of patients with ANCA vasculitis and can also be associated with subsequent disease flare. Propyl thiouracil is the most common drug cause of ANCA vasculitis, usually MPO-ANCA positive; other drugs linked to AAV include cocaine, penicillamine, hydralazine, and macrolide antibiotics (Graf, 2013).

Mimics of vasculitis

Other disorders with multisystem features that can mimic vasculitis syndromes include atheroembolic disease, antiphospholipid syndrome, and multiple myeloma. The differential diagnosis of the pulmonary renal syndrome also includes atypical pneumonia, *Hantavirus* infection, and left ventricular failure (see Chapter 72).

Overlap presentations

Rheumatoid vasculitis has become a less common association of rheumatoid arthritis but MPO-ANCA-positive MPA is recognized to occur in long-standing rheumatoid arthritis patients. ANCA positivity, usually MPO-ANCA, occurs in SLE and can be associated with a pauci-immune renal histology suggesting a true dual pathology; its significance in most SLE patients is uncertain. One-third of patients with anti-GBM disease have concurrent ANCA at the time of diagnosis and these patients have a vasculitis as well as an anti-GBM phenotype (Levy et al., 2004). Systemic vasculitis is associated with a hypercoagulative state and thrombosis, but rare patients have a dual presentation with a transferable

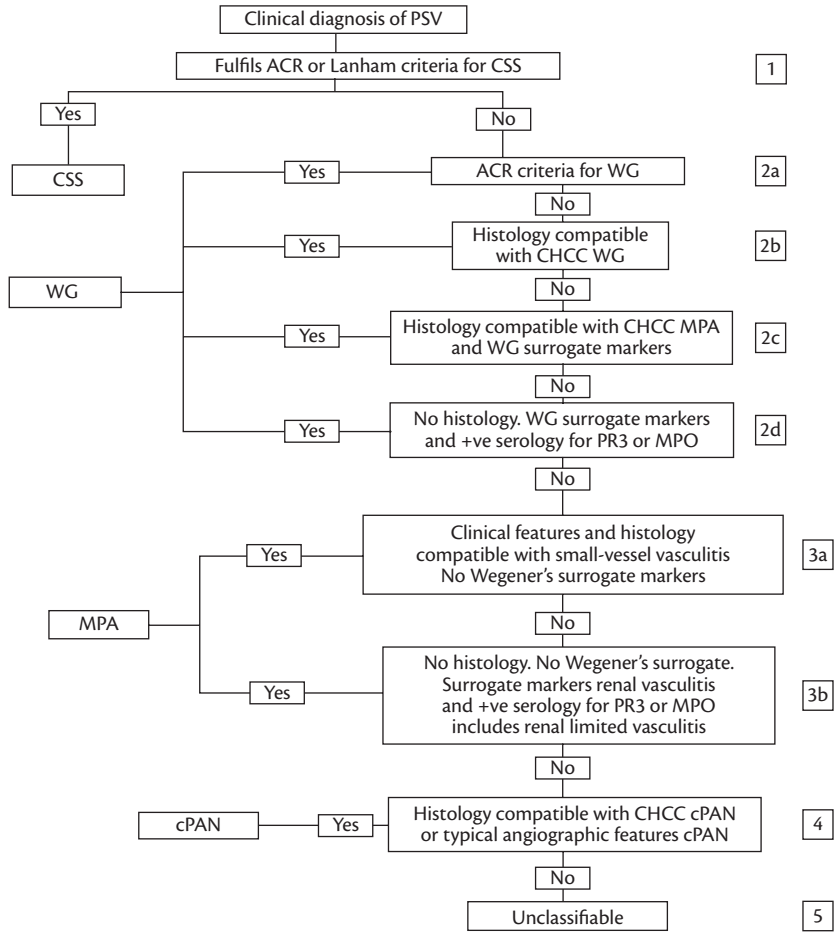

Fig. 157.4 Algorithm for the classification of a patient with ANCA-associated vasculitis or classical polyarteritis nodosa developed for the European Medicines Evaluation Agency. ACR = American College of Rheumatology; CHCC = 1993 Chapel Hill Consensus Conference; cPAN = classical polyarteritis nodosa; PSV = primary systemic vasculitides; WG = Wegener's granulomatosis, now GPA; WG surrogate markers are clinical features of the respiratory tract suggestive of WG; PR3 or MPO indicates positive PR3-ANCA or MPO-ANCA.
From Watts et al. (2007).

anticoagulant, the antiphospholipid syndrome, and are at risk of devastating gangrene and tissue necrosis due to multiple arterial thromboses.

Special considerations in children and the elderly

IgA vasculitis is the most common vasculitis in children and can occur in the first year of life. ANCA vasculitis is very rare in young children and the phenotype in older children or adolescents is similar to that seen in adults although PR3-ANCA is the predominant serotype. Kawasaki disease is a vasculitis of infants and children generally < 4 years of age that can involve large blood vessels. Autoinflammatory syndromes are an important differential diagnosis in children and may overlap because there is increased frequency of vasculitis in carriers of the Mediterranean fever gene (*MEFV*) (Ozen, 2009).

MPO-ANCA positive MPA predominates in the elderly and renal vasculitis is both more frequent and more severe in older patients. Subacute presentations may be seen with slowly progressive kidney injury; others have no focal organ manifestations and present with a 'failure to thrive'. Diagnostic delay is more common in the elderly, which is particularly problematic due to the severity of their nephritis and intolerance of vasculitis therapies.

References

Basu, N., Watts, R., Bajema, I., *et al.* (2010). EULAR points to consider in the development of classification and diagnostic criteria in systemic vasculitis. *Ann Rheum Dis*, 69(10), 1744–50.

Berden, A. E., Ferrario, F., Hagen, E. C., *et al.* (2010). Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol*, 21, 1628–36.

Cacoub, P., Renou, C., Kerr, G., *et al.* (2001). Influence of HLA-DR phenotype on the risk of hepatitis C virus-associated mixed cryoglobulinemia. *Arthritis Rheum*, 44, 2118–24.

Churg, J. and Strauss, L. (1951). Allergic granulomatosis, allergic angiitis, and periarteritis nodosa. *Am J Pathol*, 27, 277–301.

Corral-Gudino, L., Borao-Cengotita-Bengoa, M., Del Pino-Montes, J., *et al.* (2011). Overall survival, renal survival and relapse in patients with microscopic polyangiitis: a systematic review of current evidence. *Rheumatology (Oxford)*, 50, 1414–23.

De Lind van Wijngaarden, R. A., Hauer, H. A., Wolterbeek, R., *et al.* (2006). Clinical and histologic determinants of renal outcome in ANCA-associated vasculitis: a prospective analysis of 100 patients with severe renal involvement. *J Am Soc Nephrol*, 17, 2264–74.

Fahey, J. L., Leonard, E., Churg, J., *et al.* (1954). Wegener's granulomatosis. *Am J Med*, 17, 168–79.

Flossmann, O., Berden, A., de Groot, K., *et al.* (2011). Long-term patient survival in ANCA-associated vasculitis. *Ann Rheum Dis*, 70, 488–94.

Fujimoto, S., Watts, R. A., Kobayashi, S., *et al.* (2011). Comparison of the epidemiology of anti-neutrophil cytoplasmic antibody-associated vasculitis between Japan and the UK. *Rheumatology (Oxford)*, 50(10), 1916–20.

Gardner-Medwin, J. M., Dolezalova, P., Cummins, C., *et al.* (2002). Incidence of Henoch-Schonlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. *Lancet*, 360, 1197–202.

Gatenby, P. A., Lucas, R. M., Engelsen, O., *et al.* (2009). Antineutrophil cytoplasmic antibody-associated vasculitides: Could geographic patterns be explained by ambient ultraviolet radiation? *Arthritis Rheum*, 61, 1417–24.

Graf, J. (2013). Rheumatic manifestations of cocaine use. *Curr Opin Rheumatol*, 25, 50–5.

Grayson, P. C., Maksimowicz-McKinnon, K., Clark, T. M., *et al.* (2012). Distribution of arterial lesions in Takayasu's arteritis and giant cell arteritis. *Ann Rheum Dis*, 71, 1329–34.

Gregersen, J., Chaudhry, A., and Jayne, D. (2013). Rituximab for ANCA-associated vasculitis in the setting of severe infection. *Scand J Rheumatol*, 42(3), 207–10.

Hogan, S. L., Cooper, G. S., Savitz, D. A., *et al.* (2007). Association of silica exposure with anti-neutrophil cytoplasmic autoantibody small-vessel vasculitis: a population-based, case-control study. *Clin J Am Soc Nephrol*, 2, 290–9.

Holle, J. U., Herrmann, K., Gross, W. L., *et al.* (2012). Comparative analysis of different commercial ELISA systems for the detection of anti-neutrophil cytoplasm antibodies in ANCA-associated vasculitides. *Clin Exp Rheumatol*, 30, S66–9.

Hruskova, Z., Casian, A., Konopasek, P., *et al.* (2013). Long-term outcome of severe alveolar haemorrhage in ANCA-associated vasculitis: a retrospective cohort study. *Scand J Rheumatol*, 42(3), 211–14.

Hunder, G. G., Arend, W. P., Bloch, D. A., *et al.* (1990). The American College of Rheumatology 1990 criteria for the classification of vasculitis. Introduction. *Arthritis Rheum*, 33, 1065–7.

Jauhola, O., Ronkainen, J., Koskimies, O., *et al.* (2010). Renal manifestations of Henoch-Schonlein purpura in a 6-month prospective study of 223 children. *Arch Dis Child*, 95, 877–82.

Jayne, D., Rasmussen, N., Andrassy, K., *et al.* (2003). A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med*, 349, 36–44.

Jennette, J. C., Falk, R. J., Andrassy, K., *et al.* (1994). Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum*, 37, 187–92.

Jennette, J. C., Falk, R. J., Bacon, P. A., *et al.* (2013). 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum*, 65(1), 1–11.

Kain, R., Tadema, H., McKinney, E. F., *et al.* (2012). High prevalence of autoantibodies to hLAMP-2 in anti-neutrophil cytoplasmic antibody-associated vasculitis. *J Am Soc Nephrol*, 23, 556–66.

Levy, J. B., Hammad, T., Coulthart, A., *et al.* (2004). Clinical features and outcome of patients with both ANCA and anti-GBM antibodies. *Kidney Int*, 66, 1535–40.

Lyons, P. A., Rayner, T. F., Trivedi, S., *et al.* (2012). Genetically distinct subsets within ANCA-associated vasculitis. *N Engl J Med*, 367, 214–23.

Mahr, A., Guillevin, L., Poissonnet, M., *et al.* (2004). Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis, and Churg–Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. *Arthritis Rheum*, 51, 92–9.

Martorana, D., Maritati, F., Malerba, G., *et al.* (2012). PTPN22 R620W polymorphism in the ANCA-associated vasculitides. *Rheumatology (Oxford)*, 51, 805–12.

Matignon, M., Cacoub, P., Colombat, M., *et al.* (2009). Clinical and morphologic spectrum of renal involvement in patients with mixed cryoglobulinemia without evidence of hepatitis C virus infection. *Medicine (Baltimore)*, 88, 341–8.

Mohammad, A. J., Jacobsson, L. T., Mahr, A. D., *et al.* (2007). Prevalence of Wegener's granulomatosis, microscopic polyangiitis, polyarteritis nodosa and Churg–Strauss syndrome within a defined population in southern Sweden. *Rheumatology (Oxford)*, 46, 1329–37.

Ozen, S. (2009). Mutations/polymorphisms in a monogenetic autoinflammatory disease may be susceptibility markers for certain rheumatic diseases: lessons from the bedside for the benchside. *Clin Exp Rheumatol*, 27, S29–31.

Ozen, S., Ruperto, N., Dillon, M. J., *et al.* (2006). EULAR/PReS endorsed consensus criteria for the classification of childhood vasculitides. *Ann Rheum Dis*, 65, 936–41.

Pankhurst, T., Savage, C. O., Gordon, C., *et al.* (2004). Malignancy is increased in ANCA-associated vasculitis. *Rheumatology (Oxford)*, 43, 1532–5.

Patel, N., Patel, N., Khan, T., *et al.* (2011). HIV infection and clinical spectrum of associated vasculitides. *Curr Rheumatol Rep*, 13, 506–12.

Sable-Fourtassou, R., Cohen, P., Mahr, A., *et al.* (2005). Anti-neutrophil cytoplasmic antibodies and the Churg–Strauss syndrome. *Ann Intern Med*, 143, 632–8.

Sinico, R. A., Di Toma, L., Maggiore, U., *et al.* (2005). Prevalence and clinical significance of antineutrophil cytoplasmic antibodies in Churg–Strauss syndrome. *Arthritis Rheum*, 52, 2926–35.

Tadema, H., Heeringa, P., and Kallenberg, C. G. (2011). Bacterial infections in Wegener's granulomatosis: mechanisms potentially involved in autoimmune pathogenesis. *Curr Opin Rheumatol*, 23, 366–71.

Takala, J. H., Kautiainen, H., Malmberg, H., *et al.* (2008). Incidence of Wegener's granulomatosis in Finland 1981–2000. *Clin Exp Rheumatol*, 26, S81–5.

Watts, R. A., Jolliffe, V. A., Grattan, C. E., *et al.* (1998). Cutaneous vasculitis in a defined population—clinical and epidemiological associations. *J Rheumatol*, 25, 920–4.

Watts, R., Lane, S., Hanslik, T., *et al.* (2007). Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. *Ann Rheum Dis*, 66, 222–7.

Wegener's Granulomatosis Etanercept Trial Research Group (2005). Etanercept plus standard therapy for Wegener's granulomatosis. *N Engl J Med*, 352, 351–61.

Yoshioka, T., Xu, Y. X., Yoshida, H., *et al.* (1998). Deletion polymorphism of the angiotensin converting enzyme gene predicts persistent proteinuria in Henoch–Schönlein purpura nephritis. *Arch Dis Child*, 79, 394–9.

Zeek, P. M., Smith, C. C., and Weeter, J. C. (1948). Studies on periarteritis nodosa; the differentiation between the vascular lesions of periarteritis nodosa and of hypersensitivity. *Am J Pathol*, 24, 889–917.

CHAPTER 158

The patient with vasculitis: pathogenesis

Peter Heeringa and Coen A. Stegeman

Introduction

Vasculitis is a clinicopathological process defined by inflammation and damage to blood vessels. Inflammatory cell infiltration in some or all tissue layers of blood vessels cause swelling, necrosis, and disruption of vessel wall structures such as the internal elastic lamina and endothelium, compromising antithrombotic activity, vessel patency, and integrity. In addition, the inflammatory response can lead to remodelling and proliferation of vascular structures causing fibrosis and thickening of media and intima compromising the vessel lumen. Vessel wall inflammation may even extend into the tissues adjacent to the vessels causing perivascular or angiocentric inflammation. The inflammatory changes and loss of vascular functions lead to diverse sequelae such as aneurysmatic vessel dilatation, tissue ischaemia, and organ dysfunction, necrosis, and bleeding.

The clinical syndromes caused by vasculitic diseases are heterogeneous, as most forms are not—or only partially—restricted to single organs, certain vessel types, or sizes. Therefore, vasculitis usually is a systemic, multi-organ disease, although its presentation may be dominated by a single or limited number of clinical organ manifestations. In clinical practice, the most common forms of vasculitis affect the smaller blood vessels, primarily arterioles, capillaries, and postcapillary venules. Given the extensive presence of small vascular structures in the kidney, it is not surprising that renal involvement is a frequent finding in systemic vasculitic syndromes.

In the approach to the patient with suspected vasculitis it is important to realize that vasculitis may be the primary manifestation of a disease, or, alternatively, may be a secondary manifestation of another underlying disease. The distinction between primary and secondary forms of vasculitis is important as their pathophysiology may be completely different, which has prognostic and therapeutic consequences. From a pathophysiological point of view many of the secondary forms of vasculitis are associated with immune complex formation and deposition, or direct infiltration of the vascular tissue by infectious agents. For most of the primary vasculitic syndromes the pathogenesis is less clear. Classification of human vasculitic syndromes partly reflects this distinction, but syndromes are mainly categorized by the vessel size primarily involved, and the histopathological characteristics of the lesions (Table 158.1) (Jennette et al., 1994; Jennette and Falk, 1997). This chapter will discuss the pathogenesis of human vasculitis with respect to general pathogenic patterns and more disease-specific pathogenic pathways related to primary and secondary syndromes.

Immunopathological aspects of vasculitic inflammation

The pathogenesis of vasculitis is complex, and involves different mechanisms that may operate simultaneously or sequentially. It was long thought that antigen–antibody complex formation at the site of the vessel wall was the primary pathological process in all forms of vasculitis. The clinical association of infections or drugs with the development of vasculitis, in which immune depositions in the lesions could be demonstrated, substantiated this concept. From earlier work on experimental serum sickness and other models, Fauci proposed a multiple-step hypothesis for the development of necrotizing vasculitis in which antigen exposure and subsequent antibody formation would lead to circulating antigen–antibody complexes which, under specific circumstances, would be deposited in blood vessel walls causing complement activation, recruitment and activation of leucocytes, and vessel wall damage and necrosis (Fauci et al., 1978). Although operative in many secondary forms of vasculitis, the uniformity of the concept is challenged by the absence of demonstrable immune complexes in early lesions of most forms of vasculitis. In addition, in certain vasculitides the vessel wall is infiltrated by activated T lymphocytes and macrophages in the absence of both neutrophils and immune deposits.

Immune complex-mediated vasculitis

The role of immune complexes in the pathogenesis of vasculitis

The role of antigen–antibody complex formation in the development of vasculitis has been demonstrated in animal models of serum sickness and the Arthus reaction (Dixon et al., 1958). In the acute serum sickness model in rabbits, following a single intravenous dose of heterologous serum albumin, necrotizing arteritis, glomerulonephritis, and arthritis develop after 10–14 days. The lesions develop at the moment when complexes of serum albumin, antibody, and complement can be demonstrated in the circulation and in the vessel wall of arteries and glomeruli. As heterologous antigen cannot be demonstrated in the vessel wall prior to the formation of circulating and deposited immune complexes, *in situ* complex formation is not involved (Dixon et al., 1958). Chronic serum sickness with daily administration of heterologous protein results in a variety of sequelae, ranging from absence or resolution of the vasculitic process due to tolerance or rapid clearance

Table 158.1 Categories of primary and secondary vasculitic syndromes according to the predominant vessel size involved (Jennette et al., 1994; Jennette and Falk, 1997)

Large vessel vasculitis (aorta and major arterial branches)
Giant cell arteritis
Takayasu arteritis
Medium-sized vessel vasculitis (larger muscularized arteries)
Polyarteritis nodosa
Kawasaki disease
Primary granulomatous vasculitis of the central nervous system
Small vessel vasculitis (small arteries, arterioles, capillaries, venules)
ANCA-associated vasculitis:
Microscopic polyangiitis
Granulomatosis with polyangiitis
Churg–Strauss syndrome
Drug-induced ANCA-associated vasculitis
Immune-complex small vessel vasculitis:
Henoch–Schönlein purpura
Cryoglobulinaemic vasculitis
Connective tissue disease-associated vasculitis, e.g. systemic lupus erythematosus; Sjögren syndrome; rheumatoid arthritis
Hypocomplementaemic urticarial vasculitis
Behçet disease
Goodpasture syndrome
Serum sickness
Drug-induced immune-complex vasculitis
Infection-induced immune-complex vasculitis
Paraneoplastic small vessel vasculitis

of the antigen, to chronic immune complex glomerulonephritis and leucocytoclastic small vessel vasculitis (Brentjens et al., 1975; Christian and Sergent, 1976). A likely explanation for the differences observed between acute and chronic serum sickness and the inconsistent features of vasculitis in animal and human immune complex disease is that physical and immunological characteristics of complexes vary greatly. These differences in characteristics are caused by quantitative and qualitative differences both in the immune response and the antigen load, leading to different composition and size of the antigen–antibody complexes, depending on the relative amounts of antigen and antibody present. In addition, non-specific binding characteristics and size of the antigen, affinity and isotype of the antibody, and binding of complement determine the fate of these complexes (Cochrane and Hawkins, 1968; Fauci et al., 1978). Antibodies present in immune complexes are frequently of the immunoglobulin (Ig)-G isotype, but IgM and IgA have also been demonstrated in circulating and deposited immune complexes. The mere presence of circulating immune complexes is, however, not sufficient to produce vasculitis (Fig. 158.1). Experimental data have shown that vasoactive amines increasing vascular permeability derived from platelets, mast cells, and basophils are necessary for tissue deposition of immune complexes and that treatment with antihistamines prevents or attenuates vasculitis (Henson and Cochrane, 1971; McCluskey and Fienberg, 1983). The fact that deposited immune complexes and vascular lesions are preferentially found at vessel branching sites, heart valves, sites of tissue trauma, and dependent body areas, point to the necessity of certain microcirculatory circumstances for deposition and inflammation to occur (Ball and Bridges, 2002). In addition to low flow velocities, capillaries and postcapillary venules can express receptors for the fragment crystallizable (Fc) region of immunoglobulins and for complement C3b facilitating binding of complexes (Claudy, 1998) (Fig. 158.1). This explains, in part, the predilection for these vessels in immune complex-mediated vasculitis.

Inflammatory reactions following immune complex deposition

The deposition of immune complexes in vessel walls triggers a cascade of inflammatory processes (Jancar and Sanchez Crespo, 2005). Initially, the inflammatory reaction is driven by antibody-dependent complement activation which will lead to formation of chemotactic factors C3a and C5a causing recruitment, infiltration, and activation of leucocytes (Fig. 158.1). The acute phase is characterized by necrosis of endothelium and disruption of the basement membrane of capillaries and postcapillary venules due to formation of complement C5b-9 complexes and the release of lytic enzymes and oxygen radials from activated polymorphonuclear cells. In larger vessels, this process leads to infiltration of the adventitia and media and disruption of the elastic lamina. Concomitantly, the physical integrity and antithrombotic capacity of the endothelium is lost causing deposition in the vessel wall of plasma proteins and fibrin. In the model of acute serum sickness, rabbits that have been depleted of circulating complement by treatment with cobra venom do not develop vasculitis: deposited immune complexes can be demonstrated, but no complement is found and neutrophil infiltration with necrosis of vessel wall structures is absent (Henson and Cochrane, 1971). Likewise, induced neutropenia or absence of Fcγ-receptors will prevent the development of inflammation and vasculitis, pointing to the essential role of complement activation, neutrophil recruitment and Fcγ-receptor engagement with immune complexes in the early stages of the process (Sylvestre and Ravetch, 1994; Mayadas et al., 2009).

The endothelial cells are, however, by no means injured innocent bystanders of the attack. Early expression of P-selectin on endothelial cells activated by histamine, thrombin, and complement components (C5b-9) and of endothelial E-selectin by activation with tumour necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) are essential for leucocyte rolling and adherence through binding to sialyl Lewis-X and L-selectin expressed on neutrophils (Ley et al., 2007). The endothelial selectin expression peaks within hours and disappears by 24 hours, but is taken over and superseded by endothelial expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). These adhesion molecules form ligands for clusters of differentiation (CD)-11/CD18 integrins expressed on leucocytes and expression will result in strong and irreversible binding and subsequent transendothelial migration of the leucocytes (Ley et al., 2007). Activated neutrophils and monocytes will produce IL-8, which will further stimulate neutrophil migration and activation. The activated and transmigrated neutrophils will rapidly degrade

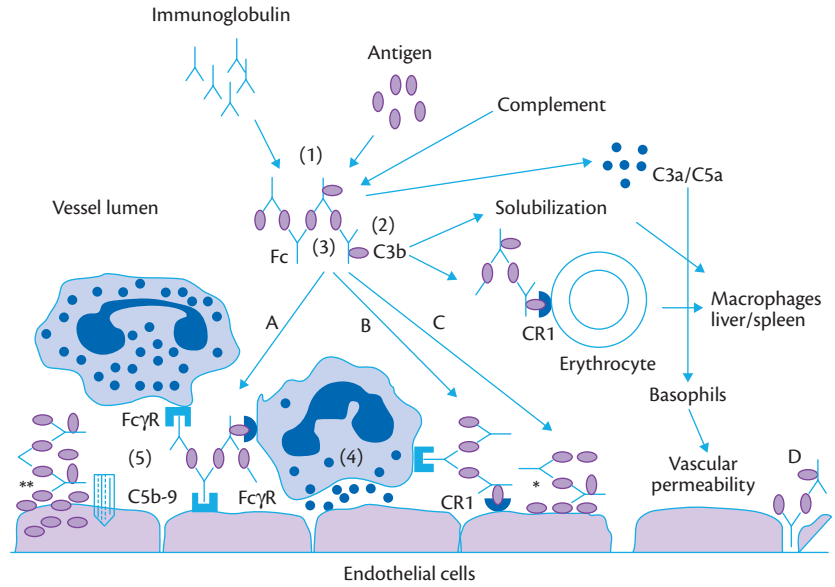

Fig. 158.1 Schematic representation of the processes involved in immune complex formation in the vessel lumen or at the endothelial cells, immune complex deposition, and initial inflammatory events in immune complex mediated vasculitis. (1) Immune complexes are formed between circulating, endothelial bound (*) or produced antigen(s) (**) and immunoglobulins. (2) Complement will be activated by the complexes through the classical or alternate pathway resulting in release of complement fragments C3a and C5a which will attract neutrophils and stimulate basophils to release vasoactive amines. C3b will bind to the immunoglobulin of the complexes and facilitate solubilization of the complex and binding to complement receptor 1 on macrophages or erythrocytes. (3) Circulating complexes not eliminated by binding to erythrocytes or macrophages can be deposited on the endothelial cells by: (A) binding of the Fc part of the complexed immunoglobulin to the Fcγ-receptors (FcγR) or (B) binding of complex bound C3b to complement receptor 1 (CR1) expressed on capillaries and postcapillary venules; (C) binding of antigen to endothelial cell membranes by charge interaction; (D) trapping of complexes with binding to subendothelial matrix due to increased vascular permeability caused by vasoactive amines. (4) Neutrophils attracted by chemotactic factors complement C3a, C5b, and locally produced cytokines (not depicted) will bind to the complexes with Fcγ-receptors (FcγR) and complement receptor 1 (CR1). Neutrophil activation and degranulation will occur with subsequent lytic and oxidative damage to the endothelial cells. In addition, bound complexes will lead to full complement activation with formation and membrane insertion of the membrane attack complex (C5b-9).

the immune complexes, which will no longer be detectable after 24–48 hours (Grunwald et al., 1997). The temporal pattern of expression of endothelial adhesion molecules in combination with secreted chemokines such as monocyte chemoattractant protein 1 (MCP-1) and regulated upon activation of normal T-cell expressed and secreted (RANTES) will change the infiltrate from predominantly neutrophils to mono- and lymphocytic.

Immune complexes in human vasculitis

Detection of immunoglobulin and complement in human leucocytoclastic vasculitis, which is the histopathological prototype of human immune complex vasculitis, provides circumstantial evidence for a role of immune complexes in the pathogenesis. These immune complexes, either deposited from the circulation or formed *in situ*, are found in Henoch–Schönlein purpura, vasculitis associated with cryoglobulinaemia, hepatitis B-associated polyarteritis nodosa, and many cases of the secondary vasculitides. The complexes involved are supposedly composed of antibodies bound to microbial antigens in case of underlying infectious diseases, autoantigens in the connective tissue diseases, and non-microbial exogenous antigens in the hypersensitivity disorders (Table 158.2). The immune complex-associated vasculitides (Table 158.1) are all associated with the presence of circulating immune complexes and vascular deposits composed of combinations of IgM, IgG, IgA, and complement C3, suggesting a role of these complexes in their pathogenesis. Definite support for a pathogenetic role for immune complexes requires the simultaneous detection of a relevant antigen and specific antibody in the circulation and the vasculitic lesions. With the exception of the small and medium sized vessel vasculitis associated with chronic hepatitis B infection, in which hepatitis B surface antigen–antibody complexes have been demonstrated in the circulation and in lesions of muscular arteries, dermal vessels, and glomeruli (Trepo et al., 1974; Michalak, 1978), the specificities of the antigens and their corresponding antibodies have not been identified in most of the cases. Although circulating immune complexes can be demonstrated in many patients with vasculitis, this finding is very aspecific as many patients with chronic hepatitis B or other infections, connective tissue diseases, or tumours have circulating immune complexes, of which only a very small fraction will develop vasculitis and glomerulonephritis.

Factors involved in immune complex formations and disease in humans

The fact that immune complex formation occurs frequently under pathological and probably also under physiological conditions, while vasculitis or other sequelae secondary to these complexes are rare, suggest that specific conditions have to be met for immune complexes to lead to immune complex vasculitis (Table 158.3). Normally, immune complexes form only transiently and are rapidly cleared from the circulation. Persistent or recurrent exposure to antigens in the circulation in combination with the presence of specific antibodies may lead to circulating complexes. Chronic

Table 158.2 Secondary vasculitides: antigens and antigen sources presumably involved in immune complex formation

Exogenous antigens
Microbial antigens:
Bacterial:
Streptococci
Staphylococci
Mycobacterium leprae
Treponema pallidum
Others
Viral:
Hepatitis B/C virus
Human immunodeficiency virus
Cytomegalovirus
Epstein–Barr virus
Others
Protozoal:
Plasmodia
Non-microbial antigens
Heterologous proteins (therapeutic monoclonal or polyclonal antibodies):
Allergens
Drugs
Tumour antigens (?)
Autologous antigens
Nuclear antigens (antinuclear antibodies)
Immunoglobulin G (rheumatoid factor, cryoglobulins)
Others

Table 158.3 Factors possibly influencing the ***in situ*** formation or deposition of circulating immune complexes and development of vasculitis

Immune complex characteristics
Concentration of circulating immune complexes
Size and composition of the immune complexes
Ratio of antigens and antibodies
Immunoglobulin class(es) and subclass(es)
Properties of the antigen
Charge of the antigen
Site of production of the antigen
Complement activating capacity
Patient characteristics
Capacity to efficiently remove circulating immune complexes
Expression and affinity of complement and Fcγ receptors on reticuloendothelial and other cells
Intact complement system
Haemodynamic conditions or factors influencing vascular permeability facilitating antigen trapping or immune complex deposition
Propensity to chronic or intermittent antigen exposure due to chronic or recurrent infection(s) or increased gastrointestinal permeability for antigens

infections such as hepatitis B and C or endocarditis, increased permeability for gastrointestinal antigens in Henoch–Schönlein purpura, and release of endogenous antigens in systemic lupus erythematosus or rheumatoid arthritis are all thought to provide these antigens and, in the presence of binding antibodies, to result in persistent or recurrent immune complex formation. On the other hand, the efficiency with which immune complexes are cleared from the circulation may be an important determinant. Activation of the classical and alternative complement pathways by the immune complexes will lead to adherence of C3b and C4b. C3b binding facilitates solubilization of immune complexes and uptake of the complex by interaction with the complement receptor type 1 (CR1) on cells of the reticuloendothelial system, especially the Kupffer cells in the sinoids of the liver. In addition, CR1 is abundantly present on the surface of erythrocytes (Fig. 158.1). Binding of immune complexes to these receptors on erythrocytes inhibits their precipitation and allows their transport to liver and spleen macrophages (Schifferli et al., 1986). In patients with systemic lupus erythematosus, a decreased density of CR1 receptors on erythrocytes has been found, resulting in decreased binding of immune complexes to erythrocytes (Miyakawa et al., 1981; Lobatto et al., 1988). The association between genetic deficiencies of complement components and immune complex diseases suggests that complement mediated clearance *in vivo* is important in preventing immune complex deposition. Immune complex uptake is also mediated by the interaction of the Fc part of complexed immunoglobulins and Fcγ-receptors on mononuclear phagocytic cells. Functional polymorphisms in the human Fcγ-receptor II and III have been described that modulate the affinity for different IgG subclasses. Associations between these functional polymorphisms and reduced clearance of immune complexes and risk for immune complex-mediated diseases such as systemic lupus erythematosus have been described (Dijstelbloem et al., 2000; Li et al., 2009).

Amounts of circulating immune complexes are often higher in patients with vasculitic disease compared to those without vasculitic disease activity, in diseases such as rheumatoid arthritis associated with vasculitis, and in Henoch–Schönlein purpura. Also in individual patients, circulating immune complex levels can fluctuate in relation with disease activity. The relation between levels of circulating immune complexes and vasculitis is, however, by no means absolute. Size and composition of the immune complexes are important: in experimental models only complexes with certain sedimentation characteristics are deposited in vessel walls (Cochrane and Hawkins 1968). In patients with drug-induced and Henoch–Schönlein purpura, the development of leucocytoclastic vasculitis coincides with the presence of large IgA and C3 containing complexes in the circulation which disappear when the patient recovers (Kauffmann et al., 1980). The size of the immune complex determines its fate as circulating and deposited IgA-immune complexes are found in both patients with IgA nephropathy and Henoch–Schönlein purpura, but these complexes are smaller in

IgA nephropathy than in Henoch–Schönlein purpura (Davin and Weening, 2001).

Specific characteristics of the immunoglobulin component or the antigen present in the complex may determine its propensity to be formed or deposited at certain vascular sites. IgA1 present in immune complexes in Henoch–Schönlein purpura has been found to be abnormally glycosylated, especially when nephritis is present (Novak et al., 2008; Suzuki et al., 2008). Likewise, in hepatitis C virus-associated mixed cryoglobulinaemia, which is present in 50% of patients with chronic hepatitis C, the small minority who cryoglobulinaemic vasculitis have more often IgG3 subclass in their complexes (Dammacco et al., 2001). Binding of endogenous antigens such as nuclear histones or bacterial antigens such as staphylococcal neutral phosphatase to (glomerular) basement membranes or endothelial cells due to charge interactions may result in deposition or *in situ* formation of immune complexes. Finally, endothelial cells may be infected by microbial agents such as cytomegalovirus or other herpes viruses, parvo B19 virus, hepatitis C virus, or intracellular growing bacteria which, in addition to direct endothelial cell damage, may result in local release of antigen and thereby formation of immune complexes with subsequent inflammatory response (Lidar et al., 2009).

Pauci-immune systemic vasculitis

In contrast to the secondary vasculitides, the primary vasculitides are, with the exception of Henoch–Schönlein purpura and vasculitis associated with essential cryoglobulinaemia, characterized by a paucity of immune deposits in the lesions. The pathogenesis of most of the primary vasculitic syndromes is unclear, but autoantibodies and T-cell mediated cellular immune reactions may be involved and will be discussed. The primary vasculitic syndromes are classified based on the size of the vessels involved, the histopathology of the lesions, and the presence of characteristic clinical symptoms. A classification scheme as well as definitions for the various vasculitic syndromes were proposed by an International Consensus Group in 1993 (Jennette et al., 1994) (Table 158.4). Potential pathogenic pathways play only a very limited role in this classification, although the association of certain small-vessel vasculitic syndromes with the presence of circulating antineutrophil cytoplasmic autoantibodies (ANCAs) is well established.

Autoantibodies in primary vasculitis

As for the large vessel vasculitides, no disease-specific autoantibodies have been described until now. Also in medium-sized vessel vasculitis, specific autoantibodies are lacking. In contrast, some forms of primary small vessel vasculitis are strongly associated with the presence of circulating ANCAs directed against proteinase 3 (PR3) and myeloperoxidase (MPO), which are cytoplasmic constituents of neutrophils and monocytes. Furthermore, anti-endothelial cell antibodies (AECAs) have been described in patients with primary vasculitis. The potential role of both groups of antibodies in the pathogenesis of vasculitis will be discussed.

Pathogenicity of ANCA in systemic vasculitis

Following the preliminary reports on the associations of antineutrophil autoantibodies with idiopathic necrotizing glomerulonephritis and vasculitis (Davies et al., 1982; Hall et al., 1984), van der Woude et al. described in 1985 the presence of autoantibodies producing a characteristic cytoplasmic immunofluorescence pattern on ethanol-fixed neutrophils in sera from a large series

Table 158.4 Names, definitions, and descriptions of common features of vasculitides adopted by the Chapel Hill Consensus Conference on the nomenclature of systemic vasculitis

Large vessel vasculitis
Giant cell (temporal) arteritis
Granulomatous arteritis of the aorta and its major branches, with a predilection for the extracranial branches of the carotid artery Features: often involves temporal artery. Usually in patients > 50 years and is often associated with polymyalgia rheumatica.
Takayashu arteritis
Granulomatous inflammation of the aorta and its major branches. Features: usually occurs in patients younger than 50 years.
Medium-sized vessel vasculitis
Polyarteritis nodosa
Necrotizing inflammation of medium-sized and small arteries without glomerulonephritis or vasculitis in arterioles, capillaries, or venules.
Kawasaki's disease
Arteritis involving large, medium-sized, and small arteries and associated with mucocutaneous lymph node syndrome. Features: coronary arteries often involved. Aorta and veins may be involved. Usually occurs in children.
Small-vessel vasculitis
Granulomatosis with polyangiitis
Granulomatous inflammation involving the respiratory tract and necrotizing vasculitis affecting small- to medium-sized vessels (e.g. capillaries, venules, arterioles, and arteries). Features: necrotizing glomerulonephritis is common. Strongly associated with the presence of anti-neutrophil cytoplasmic antibodies.
Churg–Strauss syndrome
Eosinophil-rich and granulomatous inflammation involving the respiratory tract and necrotizing vasculitis affecting small-to-medium-sized vessels and associated with asthma and eosinophilia. Features: associated with the presence of anti-neutrophil cytoplasmic antibodies.
Microscopic polyangiitis
Necrotizing vasculitis with few or no immune deposits affecting small vessels (capillaries, venules, or arterioles). Features: necrotizing arteritis involving small and medium-sized arteries may be present. Necrotizing glomerulonephritis is very common, Pulmonary capillaritis often occurs. Strongly associated with the presence of anti-neutrophil cytoplasmic antibodies.
Henoch–Schönlein purpura
Vasculitis with IgA-dominant immune deposits affecting small vessels (capillaries, venules, or arterioles) Features: typically involves skin, gut, and glomeruli and is associated with arthralgias or arthritis
Essential cryoglobulinaemic vasculitis
Vasculitis with cryoglobulin immune deposits affecting small vessels (capillaries, venules, or arterioles) and associated with cryoglobulins in serum Features: skin and glomeruli are often involved
Cutaneous leucocytoclastic angiitis
Isolated cutaneous leucocytoclastic angiitis without systemic vasculitis or glomerulonephritis.

of patients with active Wegener's granulomatosis (van der Woude et al., 1985), a disease which is now termed granulomatosis with polyangiitis (GPA) (Falk et al., 2011). Since then the close association between antibodies directed against either PR3 or MPO and granulomatosis with polyangiitis, microscopic polyangiitis, renal limited vasculitis (isolated pauci-immune necrotizing glomerulonephritis), and, to a lesser extent, Churg–Strauss syndrome has been extensively described (Falk and Jennette, 1988; Tervaert et al., 1991; Hagen et al., 1998; Savige et al., 2000). Based on a relation, albeit not absolute, between the level of these autoantibodies and disease activity of the associated vasculitic syndromes, the autoantibodies were suggested to be involved in the pathogenesis of the associated diseases (Tervaert et al., 1990; Boomsma et al., 2000; Harper and Savage, 2000; Falk and Jennette, 2002). This hypothesis also more firmly positions these diseases within the spectrum of systemic autoimmune disorders.

In humans direct evidence for a pathogenic role of ANCAS is not available. There is one intriguing case report of a neonate who developed pulmonary and renal disease after transplacental passage of MPO-ANCA suggesting development of ANCA vasculitis but most evidence is indirect and circumstantial (Bansal and Tobin, 2004). Associations between the mere presence or rises in levels of ANCAs directed against PR3 and, to a lesser extent, MPO and risk for vasculitic disease activation have been described and debated. Indeed, episodes of disease activity at diagnosis or during relapse of GPA, microscopic polyangiitis, and renal limited vasculitis are nearly always accompanied by the concomitant presence of ANCAs. However, many patients who have been treated for one of the latter diseases and are in complete remission remain ANCA positive. Clearly, the mere presence of ANCAs directed against PR3 or MPO is not sufficient for vasculitic disease activity to occur, while on the other hand ANCA is, if not directly involved in the pathogenesis, an important marker for disease activity. *In vitro* data and results from experimental animal models do, however, strongly suggest that ANCAs play a pathogenic role in small vessel vasculitic disease activity (Jennette et al., 2006) (Fig. 158.2).

Experimental and laboratory data on pathogenic effects of ANCAs

In vitro, ANCAs are able to activate neutrophils to produce reactive oxygen species and to degranulate with the release of lytic enzymes such as elastase and PR3 (Falk et al., 1990). In order to get activated by ANCA, *in vitro* neutrophils must be in a state of pre-activation ('primed'). Priming occurs in the presence of low amounts of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-8. Although low levels of PR3 may be constitutively expressed on neutrophils, priming results in enhanced expression of the target antigens of ANCAs, that is, PR3 and MPO, at the cell surface and, so, makes the antigens accessible for interaction with ANCAs (Rarok et al., 2003). *In vivo* priming may be induced by infections or colonization of the upper airways by *Staphylococcus aureus*, a potent neutrophil activator, as this was found to be associated with increased risk for relapse of ANCA-associated vasculitis (Stegeman et al., 1994). Also, most humans display constitutive expression of PR3 on neutrophils, which is highly variable between different individuals but stable within one person. A high level of constitutive PR3 expression was found to be a risk factor for PR3-ANCA-related vasculitis in one study (Witko-Sarsat et al., 1999), and a risk factor for relapse in another study (Rarok et al., 2002). More recently, aberrant expression of genes encoding granule proteins, including MPO and PR3, has been demonstrated in neutrophils from patients with ANCA vasculitis. The aberrant gene expression of MPO and PR3 involves epigenetic modifications associated with gene silencing and may contribute to increased expression of the ANCA autoantigens in patients (Ciavatta et al., 2010).

ANCA-induced neutrophil activation requires not only binding of the antibodies via their $F(ab)_2$-fragments to surface-expressed PR3 or MPO, but also interaction of their Fc-fragments with Fc-receptors on neutrophils, particularly with the FcγRIIa- and FcγRIIIb-receptor (Mulder et al., 1994; Porges et al., 1994; Kocher et al., 1998). Additional studies have shown that especially ANCAS of the IgG3 subclass interact efficiently with the FcγRIIa-receptor, and the *in vitro* capacity of sera to activate primed neutrophils was found to be closer related to the IgG3-ANCA titre than to the total IgG-ANCA titre (Mulder et al., 1995). Also, IgG isolated from sera from patients who were still PR3-ANCA positive during remission of the disease were shown to have lower amounts of IgG3-PR3-ANCA and a lesser *in vitro* neutrophil-stimulating capacity than sera collected at the time of active vasculitis. Finally, studies on Fcγ IIa- and IIIb-receptor polymorphisms in humans with ANCA-associated vasculitis have shown that certain phenotypes with a high affinity for IgG3 subclass antibodies may be associated with more severe disease and disease relapse (Edberg et al., 1997; Dijstelbloem et al., 1999; Tse et al., 2000). However, a large prospective study showed that assessment of levels of IgG3-PR3-ANCA was not better in predicting relapse of vasculitic disease activity than that of total IgG-PR3-ANCA (Boomsma et al., 2000).

Binding of membrane expressed antigen and Fcγ-receptor interaction by ANCA results in activation of neutrophils through intracellular pathways involving tyrosine phosphorylation, protein-kinase C translocation, and phosphatidyl-inositol 3-kinase activation leading to activation of neutrophil respiratory burst with release of reactive oxygen products, degranulation, and secretion of leukotrienes and inflammatory cytokines (Rarok et al., 2003). ANCA-induced neutrophil activation occurs efficiently when neutrophils are adherent to an endothelial surface, a process in which β_2-integrins are involved (Reumaux et al., 1995; Radford et al., 2001). *In vitro* studies have demonstrated that ANCA are able to induce stable adherence of rolling neutrophils to endothelial cell monolayers expressing adhesion molecules and that endothelial cells can be lysed by neutrophils in the presence of ANCA (Ewert et al., 1992; Savage et al., 1992; Radford et al., 2001). *In vivo*, this process is assumed to occur at the endothelial surface of small vessels. Indeed, activated neutrophils adherent to the endothelium are observed in renal biopsies from patients with ANCA-associated necrotizing crescentic glomerulonephritis (Brouwer et al., 1994). In addition, activated neutrophils are found within the circulation of patients with active vasculitis, and their degree of activation correlates with disease activity (Muller Kobold et al., 1998). The presence of circulating activated neutrophils may lead to trapping within dense capillary systems such as pulmonary alveoli and the glomerulus, with subsequent damage to endothelial cells (Harper and Savage, 2000). This latter concept is in agreement with the description of early vasculitic lesions found in GPA with local small vessel thrombosis and necrosis with lysed neutrophils (Donald et al., 1976; Brouwer et al., 1994).

In vitro stimulation of neutrophils by ANCA also induces the release of neutrophil extracellular traps (NETs) which are chromatin

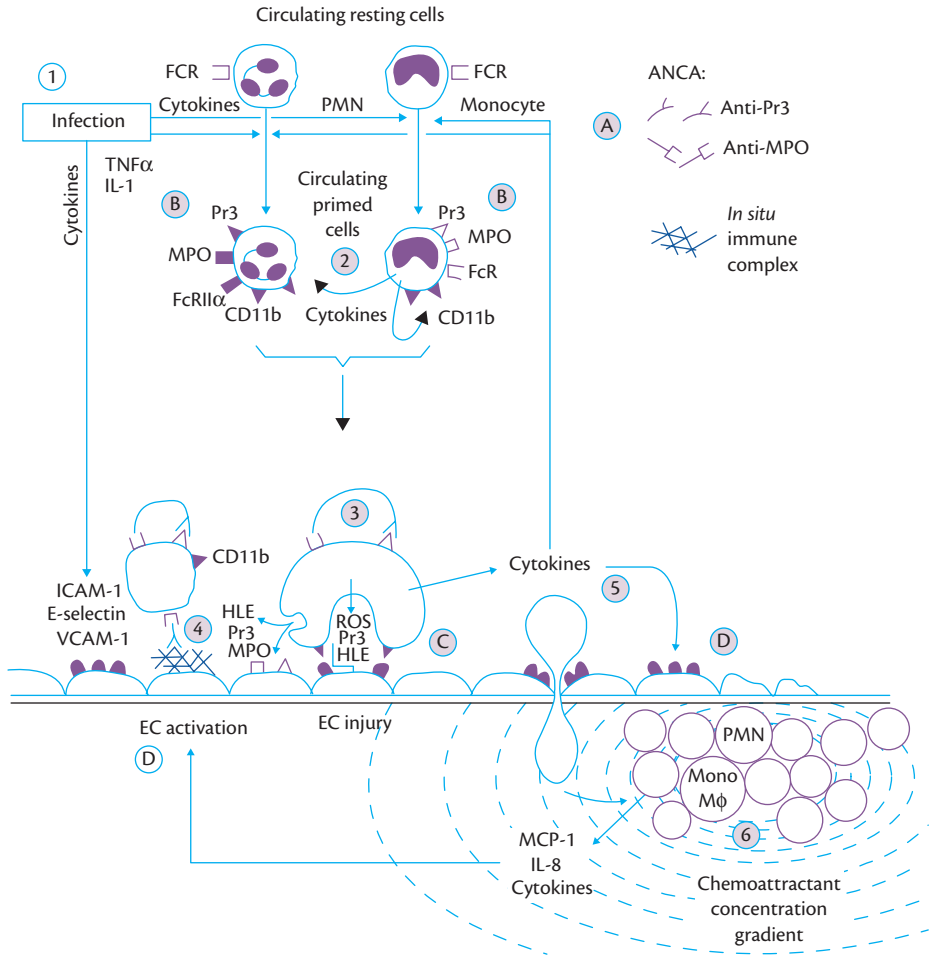

Fig. 158.2 Schematic representation of the immune mechanisms involved in the pathophysiology of ANCA-associated vasculitides. (1) Cytokines released due to (local) infection cause upregulation of adhesion molecules on the endothelium and priming of neutrophils and/or monocytes. (2) Circulating primed neutrophils and/or monocytes express the ANCA antigens on the cell surface. (3) Adherence of primed neutrophils and/or monocytes to the endothelium, followed by activation of these cells by ANCA. Activated neutrophils and/or monocytes release reactive oxygen species (ROS) and lysosomal enzymes, which leads to endothelial cell injury and eventually to necrotizing inflammation. (4) Degranulation of proteinase 3 (Pr3) and myeloperoxidase (MPO) by these ANCA activated neutrophils and/or monocytes results in endothelial cell activation, endothelial cell injury or even endothelial cell apoptosis. Furthermore, bound Pr3 and MPO serve as planted antigens, resulting in *in situ* immune complexes, which in turn attract additional neutrophils. (5) ANCA-induced monocyte activation leads to production of monocyte chemoattractant protein-1 (MCP-1) and interleukin 8 (IL-8) by these cells. The release of these chemoattractants by these cells amplifies monocyte and neutrophil recruitment possibly leading to granuloma formation. (6). (A) to (D) represent the four prerequisites for endothelial cell damage by ANCA; (A) the presence of ANCA, (B) expression of the target antigens for ANCA on primed neutrophils and monocytes, (C) the necessity of an interaction between primed neutrophils and endothelium via β_2-integrins, and finally, (D) activation of endothelial cells.
From Muller Kobold et al. (1999).

structures loaded with granule derived antimicrobial proteins including MPO and PR3 (Kessenbrock et al., 2009). Interestingly, NET formation was also detected in affected glomeruli from patients with ANCA glomerulonephritis. Formation of NETs can induce injury to endothelial cells directly but may also be involved in the perpetuation of the autoimmune response by providing a platform for presenting the autoantigens to the immune system.

Neutrophils are not the only cells expressing the ANCA target antigens PR3 and MPO. Monocytes are known to contain and express PR3 and MPO, and can interact with ANCA. *In vitro*, activation of monocytes by ANCA has been shown with release of IL-8 and MCP-1 (Casselman et al., 1995; Ralston et al., 1997). Priming with TNF-α is not a prerequisite for monocyte activation by ANCA, but does lead to stronger activation. Infiltration by activated monocytes in crescentic glomerulonephritis associated with ANCA-related vasculitis has been shown. It is mediated by chemotaxis induced by fibrin and MCP-1 produced within the glomeruli and VCAM-1 expression on glomerular endothelium (Rastaldi et al., 1996).

Upon degranulation from activated neutrophils, PR3 and MPO bind to endothelial cells and can be targeted by ANCA leading to local *in situ* immune complex formation and complement dependent damage (Ballieux et al., 1994). In addition, PR3 and MPO may also have direct effects on endothelial cells by promoting apoptosis and enhancing vascular permeability, endothelial cell detachment, and expression of adhesion molecules, thereby increasing local inflammation and damage (Taekema-Roelvink et al., 2001; Yang et al., 2001).

ANCAS directed against PR3 may reversibly bind to PR3 and interfere with the binding of PR3 with its main inhibitor

α1-antitrypsin. This may lead to diminished clearance of PR3. *In vitro* this inhibition of PR3-α1-antitrypsin complexation can be demonstrated and was found to correlate with disease activity (Dolman et al., 1993). Also, congenital α1-antitrypsin deficiency with an overrepresentation of PiZ and PiZZ alleles is associated with PR3-ANCA-related vasculitis, or to a more severe outcome of the disease. This suggests that interference with the regulation of the enzymatic activity of PR3 may be involved in the pathogenesis of PR3-ANCA-related vasculitis (Harper and Savage, 2000). Likewise, binding to MPO of MPO-ANCA may interfere with the physiological inhibition of MPO by ceruloplasmin binding.

Animal models of ANCA-associated vasculitis

An important step in proving the direct pathogenic potential of MPO-ANCA *in vivo* has been the development of animal models of anti-MPO antibody mediated vasculitis. During the 1990s, several rodent models were developed attempting to elucidate the pathogenic effects of anti-MPO antibodies (reviewed in Heeringa et al., 1998). These models involved induction of autoimmunity with the polyclonal B cell stimulator mercuric chloride, planting of ANCA antigens within the kidney by renal perfusion, or focusing the immune response to MPO on the kidney by administration of subnephritogenic doses of heterologous antiglomerular basement membrane antibodies (Heeringa et al., 1998). Although these models demonstrated the ability of anti-MPO antibodies to aggravate mild renal injury, the induction of vasculitis and glomerulonephritis was either dependent on the presence of immune complexes in the kidney, or the anti-MPO response was part of a broad, non-specific, antibody profile. Therefore, these approaches implicated ANCA as a co-factor but not as an independent cause of disease and do not accurately model the pathology of MPO-ANCA-associated vasculitis in humans.

A major breakthrough in this area came in 2002 when direct evidence was provided that MPO-ANCA causes pauci-immune glomerulonephritis and vasculitis in mice (Xiao et al., 2002). In this model, MPO gene-deficient mice were employed to circumvent tolerance to MPO and allowing induction of an anti-MPO immune response. Adoptive transfer of anti-MPO positive splenocytes into immune deficient recombinase activating gene deficient mice that lack functioning T and B cells, resulted in circulating MPO-ANCA accompanied by the development of crescentic glomerulonephritis and vasculitis. Importantly, systemic administration of anti-MPO antibodies alone into wild-type C57Bl6 mice also induced pauci-immune, focal necrotizing crescentic glomerulonephritis and systemic vasculitis providing direct evidence that MPO-ANCA are pathogenic (Fig. 158.3).

Soon after this mouse model was developed, a rat model of systemic anti-MPO associated vasculitis was reported (Little et al., 2005). Upon immunization with purified human MPO in complete Freund's adjuvant, Wistar Kyoto (WKY) rats develop high titres of MPO-ANCA that cross-reacted with rat MPO. The induction of the anti-MPO response was accompanied by the development of haematuria, albuminuria, and small vessel vasculitis in the kidneys and lungs. The histopathological features of both the mouse and rat model resemble to a large extent those in human ANCA vasculitis. Similar to the pauci-immune nature of the glomerular capillary lesions observed in human ANCA-associated glomerulonephritis, only limited deposits of immunoglobulins and complement factors are detected in affected glomeruli of mice and rats with anti-MPO mediated glomerulonephritis.

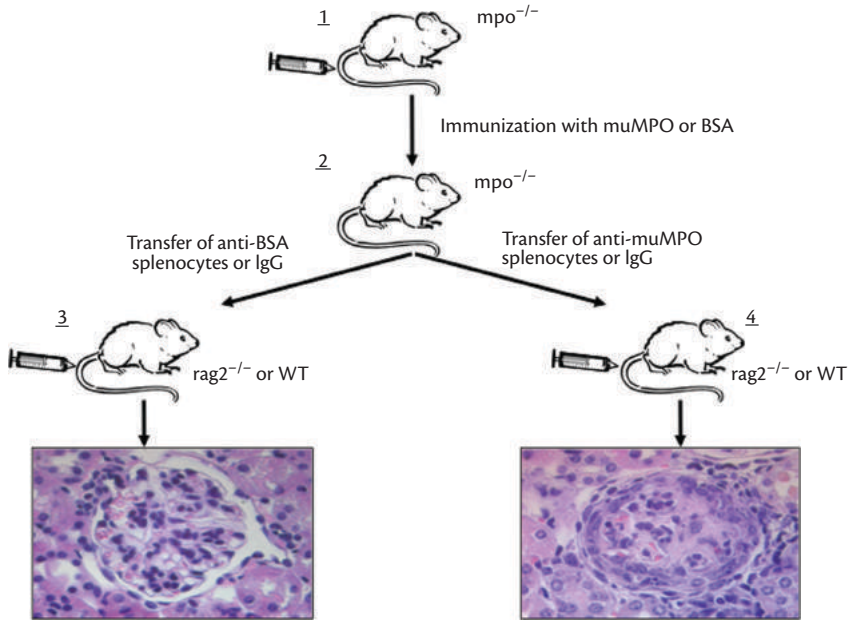

Fig. 158.3 Mouse model of anti-MPO associated glomerulonephritis and vasculitis. (1) Myeloperoxidase deficient mice (MPO −/−) are immunized with murine MPO (muMPO) or bovine serum albumin (BSA, control). (2) Splenocytes and IgG are obtained from immunized MPO −/− mice. (3) Adoptive transfer of BSA+ splenocytes into RAG2 −/− or passive transfer of BSA+ IgG into RAG2 −/− or wild−type mice induces no disease. (4) Adoptive transfer of muMPO+ splenocytes into RAG2 −/− or passive transfer of muMPO+ IgG into RAG2 −/− or wild-type mice induces vasculitis and necrotizing crescentic glomerulonephritis. Pictures represent glomerular lesions as observed 6 days after passive transfer into wild-type mice of anti-BSA IgG and anti-MPO IgG, respectively.
From Heeringa and Tervaert (2004).

Both the mouse and rat model of MPO-ANCA vasculitis convincingly demonstrate the pathogenic potential of MPO-ANCA but also have their limitations (Heeringa and Little, 2011). Essentially, both cannot be regarded as genuine autoimmune models, as they rely on active immunization in adjuvants. Also, in the mouse model, the MPO deficient mouse has never been exposed to any MPO molecule before and there is no requirement for breaking tolerance.

In contrast to MPO-ANCA, attempts to develop PR3-ANCA vasculitis models have been less successful. Using an approach similar to the murine MPO-ANCA model, immunization of PR3/elastase double knock-out mice with murine recombinant PR3 induced high-titre anti-PR3 antibodies (Pfister et al., 2004). Upon systemic administration into wild-type mice, these anti-PR3 antibodies aggravated subcutaneous panniculitis induced by intradermal injection of TNFα. However, in contrast to anti-MPO antibodies, the presence of circulating anti-PR3 antibodies alone did not cause vasculitis. In a model involving transfer of splenocytes from recombinant Pr3 immunized mice into immunodeficient, non-obese, diabetic—(NOD)—severe combined immunodeficiency (SCID) mice high levels of anti-PR3 could be detected associated with the development of necrotizing glomerulonephritis (Primo et al., 2010). These experiments support a possible pathogenic effect of anti-PR3 antibodies. However, transfer of splenocytes into immune deficient mice causes substantial immune deposits in the kidney rendering this approach less representative for human pauci-immune vasculitis. Therefore, as convincing animal models are unavailable, direct evidence for a pathogenic role of anti-PR3 autoantibodies is lacking.

The reasons for the discrepancy in pathogenic potential between anti-PR3 and anti-MPO antibodies in animal studies are unknown but may be due to species differences in antigen expression levels and differences in physicochemical properties of the antigens themselves (Jenne et al., 1997). For example, the isoelectric points of murine and human Pr3 are far less than that of MPO (isoelectric points of ~ 7 and > 10, respectively) which theoretically could result in differential interactions of the antigens with negatively charged cell structures.

Inflammatory effector mechanisms in ANCA-mediated vasculitis: insights from animal models

The rodent models of MPO-ANCA-mediated vasculitis have been used to unravel the inflammatory effector mechanisms involved in disease induction and progression (Heeringa and Little, 2011). In the mouse model, neutrophils have been identified as the main effector cells, as neutrophil depletion completely prevented vasculitis induction upon injection of anti-MPO antibodies (Xiao et al., 2005). In the same model, co-administration of anti-MPO antibodies and lipopolysaccharide severely aggravated glomerulonephritis development, which is consistent with the contention that following infection, pro-inflammatory stimuli and MPO-ANCA synergize to cause vasculitic manifestations (Huugen et al., 2005).

Intravital microscopy of the mouse cremasteric microvasculature has been used to investigate the very early events in the interaction of neutrophils with the endothelium. In the presence of a local inflammatory stimulus, anti-MPO antibodies reduced neutrophil rolling but at the same time promoted adhesion and transendothelial migration of leucocytes (Nolan et al., 2008). The MPO-ANCA-mediated effects on neutrophil-endothelium interactions were found to be dependent on β2-integrins and Fcγ-receptors. The ability of anti-MPO antibodies to increase leucocyte adhesion to and transmigration through the endothelium is also supported by intravital microscopy studies in the MPO-ANCA rat model (Little et al., 2005).

As described earlier, a central event in the pathogenesis of ANCA-associated vasculitis is the ability of ANCA to activate neutrophils which is greatly facilitated by minor pro-inflammatory stimuli such as TNFα that prime the neutrophil to interact with ANCA. Simultaneous engagement of the $F(ab)_2$ portion of ANCA with their antigens on the cell surface and interaction of the Fc part of the autoantibody with Fc receptors triggers the various signalling cascades that lead to neutrophil activation. Using the MPO-ANCA rodent models interventions aimed at blocking the proinflammatory effects of TNFα have been tested and were found to ameliorate disease severity (Huugen et al., 2005; Little et al., 2006). Additional interventions have focused on inhibition of signalling pathways involved in ANCA-mediated neutrophil activation. Transplantation of bone marrow from PI3Kγ deficient mice into irradiated MPO-immunized MPO-/-mice prevented glomerulonephritis development. Similar effects were observed in mice transplanted with wild type bone marrow upon oral treatment with a PI3Kγ specific inhibitor (Schreiber et al., 2010). Another signalling pathway implicated in ANCA-mediated neutrophil activation is the P38 mitogen-activated protein kinase (MAPK) pathway. *In vitro*, blocking P38 MAPK inhibits ANCA induced neutrophil activation and biopsy studies have shown that the P38MAPK pathway is activated in glomerular lesions of ANCA vasculitis patients (Kettritz et al., 2001; Polzer et al., 2008). In the anti-MPO mouse model, oral treatment with a P38MAPK inhibitor reduced macrophage influx and glomerular crescent formation but did not prevent disease development (van der Veen et al., 2011).

Alternative complement pathway activation in ANCA-associated vasculitis

It has generally been assumed that the complement system is not involved in the pathogenesis of ANCA-associated vasculitis because there is a paucity of immune deposits in the vasculitic lesions and circulating complement levels in patients are unperturbed. However, in several biopsy studies a certain degree of immune complex deposition in skin lesions as well as in early lesions in the kidney has been reported (reviewed in Van Timmeren et al., 2009). Importantly, compelling evidence from animal model studies now indicates a pivotal role for alternative pathway complement activation in disease pathogenesis. In the MPO-ANCA mouse model, mice lacking complement component C5, its receptor C5aR or the alternative pathway component Factor B, were completely protected from disease induction (Xiao et al., 2007; Schreiber et al., 2009). Moreover, treatment with a C5 inhibiting antibody markedly attenuated glomerulonephritis development (Huugen et al., 2007). The mechanisms responsible for ANCA-mediated complement activation have not been fully delineated yet. However, upon activation neutrophils release factors, including reactive oxygen radicals and proteases that can activate the alternative complement pathway. In addition, neutrophils themselves are a source of various complement factors including C3 and components that are unique to the alternative complement pathway, factor B and properdin, suggesting that at sites of neutrophilic inflammation all components necessary to activate the alternative pathway of complement are present (Van Timmeren et al., 2009). *In vitro*, incubation of normal

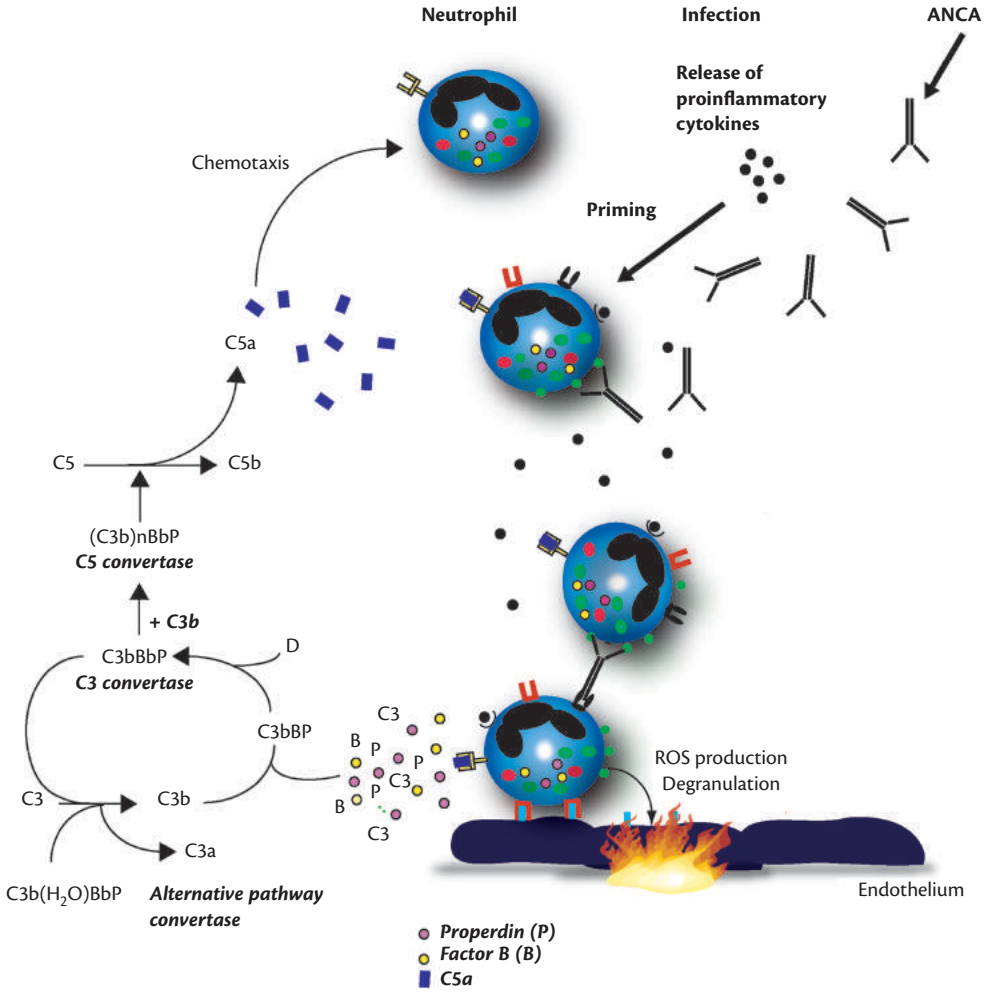

Fig. 158.4 Proposed model for the involvement of alternative pathway complement activation in ANCA-mediated vascular inflammation. Neutrophils are primed by cytokines to express ANCA antigens (MPO and Pr3) at the cell surface. Primed neutrophils adhere to susceptible endothelium and ANCA antibodies interact with the ANCA antigens, resulting in neutrophil activation. The ANCA-activated neutrophils release factors (e.g. properdin, factor B, proteases, oxygen radicals, and MPO) that can directly damage the endothelium but also activate the alternative complement pathway with the generation of the powerful neutrophil chemoattractant C5a. This mechanism of complement activation amplifies neutrophil influx and activation eventually culminating in the severe necrotizing vascular inflammation as observed in ANCA-associated vasculitis.
From Van Timmeren et al. (2009).

human serum with supernatants of ANCA activated neutrophils leads to complement activation as evidenced by the generation of C3a and C5a (Xiao et al., 2007). C5a itself is a potent chemoattractant for neutrophils, primes neutrophils for ANCA-mediated neutrophil activation and induces release of properdin. Based on these observations, a mechanism emerges in which ANCA-mediated neutrophil activation causes the release of factors that activate the alternative complement pathway initiating an inflammatory amplification cascade that ultimately results in severe necrotizing vasculitis (Van Timmeren et al., 2009) (Fig. 158.4).

Immunogenesis of ANCA-associated vasculitis

Why immunological tolerance to PR3 and MPO is broken in ANCA-associated vasculitis is unknown. Specific events that trigger the autoimmune response have not been identified and it is generally believed that the initiation of autoimmunity involves a complex interplay between genetic, environmental and, immunological factors. At least three hypotheses have been proposed to explain the generation of ANCA.

The first hypothesis posits that the initial immune response is not against PR3 but against an epitope on an antisense peptide that is complementary to a peptide on PR3 (cPR3) (Pendergraft et al., 2004). The immune response against cPR3 results in the development of anti-cPR3 antibodies, which in turn could trigger the generation of anti-PR3 antibodies via the idiotype-anti-idiotype pathway. This theory is based on the serendipitous observations that PR3-ANCA-positive vasculitis patients not only had circulating anti-PR3 antibodies but also harboured antibodies to antisense complementary PR3 peptides (Pendergraft et al., 2004). The source of the cPR3 peptides is unknown but could be either endogenous or infectious microorganisms that display peptides that mimic cPR3.

A second theory proposes that molecular mimicry between peptides on fimbriated pathogens and human lysosomal associated membrane protein 2 (LAMP-2) triggers autoimmunity to LAMP-2

and causes the development of pauci-immune necrotizing glomerulonephritis (Kain et al., 2008). Besides the canonical ANCA directed against PR3 and MPO, a high prevalence of antibodies directed against LAMP-2 was detected in patients with active pauci-immune focal necrotizing glomerulonephritis. A LAMP-2 peptide was found to have 100% homology to FimH, an adhesin expressed on certain Gram-negative bacteria. Also, in rats immunized with FimH antibodies to rat and human LAMP-2 could be detected in conjunction with the development of pauci-immune necrotizing glomerulonephritis suggesting that anti-LAMP2 antibodies are pathogenic.

The two theories on the origin of the autoimmune response in ANCA-associated vasculitis described above have been debated and are controversial because follow-up studies from other laboratories could not completely confirm these observations (Preston and Falk, 2011; Tadema et al., 2011).

Finally, it has been suggested that ANCAs are part of the repertoire of natural autoantibodies (NAAs) since MPO- and PR3-specific NAAs have been detected in the plasma of healthy individuals at low levels (Cui et al., 2010). The NAAs against MPO were found to be of significantly lower titre and avidity for MPO compared to MPO-ANCA derived from vasculitis patients which could explain the non-pathogenic nature of NAAs in healthy individuals (Xu et al., 2011). These observations clearly require confirmation in larger cohorts but do suggest that dysregulation of the NAA-producing B cells may underlie the development of high-affinity autoantibodies resulting in autoimmune pathology.

AECAs in primary systemic vasculitis

Nearly four decades ago antibodies which specifically stained the vascular endothelium on mouse kidney sections as a substrate were reported for the first time (Lindqvist and Osterland, 1971). Later, these AECAs have been described in the primary vasculitides but also in secondary vasculitides, connective tissue diseases, and a variety of other (inflammatory) disorders (Belizna et al., 2006). AECAs represent a heterogeneous group of antibodies and their target antigens are, generally, poorly characterized. AECAs are generally detected by enzyme-linked immunosorbent assays (ELISAs) using as a substrate cultured human umbilical vein endothelial cells (HUVECs). AECAs react with different endothelial antigens ranging in molecular weight from 25 to 200 kDa. However, most of the antigens recognized by AECAs are not specific for endothelial cells as these antibodies also react with fibroblasts or peripheral blood mononuclear cells (Belizna et al., 2006). Whether the antigens recognized by AECAs on endothelial cells are expressed constitutively or upon activation of endothelial cells or are so called 'planted' antigens that adhere to endothelial cells by charge interaction, is unclear. Potential candidate antigens that indeed can adhere to endothelial cells and to which antibodies can be detected in AECA assays are β_2-glycoprotein and deoxyribonucleic acid (Belizna et al., 2006). Using a proteomics approach on HUVEC extracts, peroxiredoxin 2 has been identified as a possible target for AECAs in systemic vasculitis (Karasawa et al., 2010).

AECAs have been reported in extremely varying frequencies in patients with all forms of primary systemic vasculitis, but are clinically of little value due to lack of disease specificity. Possibly, full characterization of the target antigens will improve their diagnostic significance. However, serial levels of AECAs have been reported to correlate with disease activity in primary small vessel vasculitis and Kawasaki disease, which may point to a pathogenetic role, although the possibility that AECAs are a result and not a cause of endothelial cell injury clearly exists (Leung et al., 1986; Chan et al., 1993).

In vitro, AECAs have been shown to mediate complement and cellular-dependent cytotoxicity against cultured endothelial cell monolayers (Savage et al., 1991; del Papa et al., 1992). Binding of AECAs to endothelial cells also results in endothelial cell activation with expression of IL-1, -6, and -8, MCP-1, ICAM-1, and VCAM-1 (Del Papa et al., 1996; Carvalho et al., 1999). Other *in vitro* effects of AECAs on endothelial cells that have been reported are promotion of thrombotic events with increased production of tissue factor and von Willebrand factor, shedding of heparan sulphate, and inhibition of prostacyclin production (Frampton et al., 1990; Savage et al., 1991; Lindsey et al., 1994; Ihrcke and Platt, 1996). Finally, AECAs from patients with systemic vasculitis may induce apoptosis of endothelial cells (Bordron et al., 1998).

A definite animal model supporting a pathophysiological role for AECAs in the development of vasculitis has not yet been discovered. However, injection of antibodies to known endothelial antigens such as angiotensin-converting enzyme or factor VIII von Willebrand complex induces lung vasculitis and glomerulonephritis in rabbits and rats (Belizna et al., 2006). Taken together, these findings suggest that AECAs may have a direct role in the induction of endothelial damage but a definite role in the pathogenesis of vasculitis is unproven.

T-cell mediated immune responses in primary vasculitis

ANCA-associated vasculitis

T-cell mediated immune responses are suggested to be important in different forms of primary vasculitis and substantial progress has been made in characterizing these responses (Berden et al., 2009). Human biopsy material and animal models suggest that in small vessel vasculitis the vasculitic lesions develop over time from a predominantly neutrophilic lesion into a lesion in which activated mononuclear cells (i.e. monocytes/macrophages and T cells) predominate. Activated T cells can be clearly demonstrated in lesions of human necrotizing small vessel vasculitis in renal, lung, and nasal biopsies. In addition, the occurrence of granulomas in GPA and Churg–Strauss syndrome indicate the activation of these cell types. The activated T cells in these types of vasculitides are predominantly $CD4^+$ with a Th1 dominant profile characterized by high interferon gamma (IFN-γ) and low IL-4 and -5 production (Csernok et al., 1999; Cunningham et al., 1999). Patients with ANCA-associated vasculitides also show activated T cells in their peripheral blood, a pattern that persists even when the disease is in remission (Ludviksson et al., 1998; Popa et al., 1999). This pattern of activated T cells is also reflected by increased serum levels of soluble T-cell activation markers (Stegeman et al., 1993). Finally, antigen-specific T cells recognizing the ANCA antigens PR3 and MPO have been identified in the peripheral blood of patients with ANCA-associated vasculitis (Brouwer et al., 1994; Griffith et al., 1996; Popa et al., 2002). However, no clear association between these findings and disease activity has been found, nor have these antigen-specific cells been isolated from vasculitic lesions. As the predominant type of T cells is $CD4^+$, it is possible that these cells mainly are responsible for antigen specific B-cell help, a concept entirely compatible with the IgG-class high-affinity type of autoantibodies that ANCA are.

More recent work provides additional evidence for an important role of T-cell-driven immune responses in ANCA-associated vasculitis and has documented changes in the frequency and function of circulating T-cell populations (Berden et al., 2009). Proportions of $CD4^+$ effector memory T cells ($CD4^+$TEM) were shown to be increased in ANCA vasculitis patients in remission but decreased during active disease (Abdulahad et al., 2006). At the same time, analysis of the urinary sediment showed a remarkable increase in $CD4^+$TEM during active disease again decreasing or even disappearing during disease remission (Abdulahad et al., 2009). These changes are compatible with migration of $CD4^+$TEM to inflamed tissues including the kidney. Interestingly, $CD4^+$TEM display features similar to natural killer (NK) cells including surface expression of the receptor NKG2D and cytotoxic capacity. The NKGD2 molecule is an activating C-type lectin-like receptor and one of its ligands is the major histocompatibility complex class-I chain related molecule A (MICA). MICA is expressed upon cellular stress and injury in various cell types and has been detected in peritubular endothelium and glomerular epithelium in renal lesions of ANCA-associated vasculitis patients (Capraru et al., 2008). The interaction between NKGD2 and MICA has been demonstrated to mediate cytotoxicity *in vitro* suggesting that such a mechanism may contribute to tissue injury in ANCA-associated vasculitis (Abdulahad et al., 2009).

Natural T regulatory cells (Treg) are a subset of $CD4^+$ T cells that express high levels of CD25 and the unique transcription factor FOXP3. These cells are important in controlling immune system activation and maintaining tolerance to self-antigens. Reductions in the numbers of Treg cells or defects in their suppressive function have been demonstrated in several autoimmune diseases. In patients with GPA, numbers of Treg cells are unchanged but Treg cells fail to suppress T-cell proliferation in response to PR3 indicating functional impairment (Abdulahad et al., 2007; Morgan et al., 2010). In contrast, in microscopic polyangiitis patients Treg cell function is not defective but diminished FOXP3 levels have been detected (Chavele et al., 2010). Thus, disturbances in the proportions and/or function of Treg cells may contribute to the initiation and persistence of the autoimmune response in ANCA-associated vasculitis.

Th17 cells are a recently defined Th cell subset characterized by secretion of IL-17A and other cytokines including IL17F, IL-21, and IL-22. Th17 cells have gained considerable interest as an important pathogenic effector subset contributing to inflammation and autoimmunity. In ANCA vasculitis patients in remission, a skewing towards Th17 cells and a relative increase in auto-antigen specific Th17 cells following *in vitro* stimulation has been reported suggesting a role for Th17 cells in autoantibody production (Abdulahad et al., 2008). Moreover, increased serum IL-17A levels have been detected in ANCA vasculitis patients compared to healthy controls (Nogueira et al., 2010). Of particular interest for ANCA-associated vasculitides are the pro-inflammatory effects of IL-17A which induces mobilization, recruitment, and activation of neutrophils and stimulates macrophages to produce IL-1β and TNF-α (Kitching and Holdsworth, 2011). These pro-inflammatory effects suggest that IL-17A may directly contribute to the acute vascular inflammatory response in ANCA-associated vasculitis which is supported by the observation that IL-17A deficient mice are protected from disease development in a model of autoimmune anti-MPO-mediated glomerulonephritis (Gan et al., 2010). The association of PR3-ANCA-positive GPA with increased nasal carriage of *Staphylococcus aureus* seems to further strengthen the importance of Th17 responses in disease pathogenesis. Intranasal injection of peptidoglycans from *S. aureus* has been shown to skew the immune response towards a Th17 phenotype whereas *S. aureus* derived superantigens can induce expansion and IL-17 production in $CD4^+$ T cells (Abdulahad et al., 2009). This suggests that *S. aureus* infection may drive the Th17 response in ANCA-associated vasculitis.

An emerging concept that may underlie the aberrant Th-cell polarization in ANCA-associated vasculitis is T-cell lineage plasticity. In a proinflammatory environment, functional Treg cells have been demonstrated to convert into IL17 producing Th17 cells which could explain the observed defective Treg function in conjunction with Th17 skewing in ANCA-associated vasculitis (Abdulahad et al., 2011).

Giant cell arteritis and other primary vasculitides

In the large vessel vasculitides, a role for T-cell mediated immune response is also suggested although most evidence is circumstantial. The most common form is giant cell arteritis, its name derived from the presence of many Langerhans giant cells in the lesions. The vasculitis, involving large muscular arteries with a predilection for the temporal artery, is characterized by a granulomatous inflammatory reaction within the medial vessel wall with accumulation of $CD4^+$ T-cells, macrophages, multinucleated giant cells, and disruption of the internal elastic lamina (Weyand and Goronzy, 1999). The predilection of the disease for white people from northern European countries and northern areas in the United States suggests that besides environmental factors, genetic factors are also involved. HLA-typing has shown an association with class II HLA-DR4 and with HLA-DRB1 alleles, particularly with DRB*0401, *0404, and *0408, which points to the involvement of antigen-specific T lymphocytes in the pathogenesis and is in line with the predominance of $CD4^+$ T-cell involvement seen in this disease. Clonally expanded T-cells, as detected by T-cell receptor β chain analysis in biopsies from involved temporal arteries, have been found at different sites in the biopsy but not in the peripheral blood. *In vitro* some of these T-cell clonotypes showed proliferation when incubated with monocytes pulsed with temporal artery extracts from patients but not with extracts from control temporal arteries (Brack et al., 1997). These data point to an (auto) antigen-specific T-cell response in which a modified antigen present in diseased arteries may be involved. Actinically degenerated elastic tissue has been suggested as the relevant autoantigen, although characterization of the precise antigenic structures and their modification(s) has not been accomplished.

Viral or bacterial infections have also been implicated in triggering the disease as temporal associations of giant cell arteritis with parvovirus B19 infection, parainfluenza type I infection, influenza vaccination, and *Chlamydia pneumoniae* infection have been described and parvovirus B19 and *Chlamydia pneumoniae* DNA have been found in diseased arteries (Levine and Hellmann, 2002). Analysis of cytokine patterns in biopsies from patients with giant cell arteritis shows mRNA expression of IL-2 and IFN-γ as well as IL-1β but not of IL-10 (Weyand et al., 1997). Similar to the ANCA-associated small vessel vasculitides, the Th17 axis has been implicated in giant cell arteritis (Deng et al., 2010). Proportions of

Th17 cells were substantially increased in the peripheral blood of untreated giant cell arteritis patients and accumulate in vascular lesions. In both compartments increased frequencies of IFN-γ producing Th1 cells were detected as well. Interestingly, following corticosteroid therapy of the same patients, Th1 cells persisted whereas Th17 cells were almost undetectable in lesions and peripheral blood. Thus, in giant cell arteritis at least two Th cell lineages are activated suggesting that multiple triggers are involved in disease pathogenesis (Weyand et al., 2011).

In contrast to giant cell arteritis, the infiltrate in Takayasu arteritis predominantly consists of $CD8^+$ and natural killer (NK) T cells, which is in line with the finding that the disease is associated with HLA class I antigens of the B-locus and not with class II antigens. Moreover, the T-cell receptor α- and β-chains of these infiltrating cells show a restricted use of variable genes, which is similar within the lesions (Seko, 2002). These data suggest that the infiltrating cells are directed against a specific antigen.

As some have found an association with responses to *Mycobacterium tuberculosis*, especially heat-shock protein (HSP)-65, the hypothesis that contact with mycobacteria may elicit the disease in susceptible individuals has been put forward. Further evidence for a cytotoxic T-cell response in the pathogenesis of Takayasu arteritis is found in an animal model in pigs in which granulomatous large vessel vasculitis strongly resembling Takayasu arteritis is induced by a cytotoxic T-cell response following gene transfer of a foreign HLA class I antigen to the vessel (Nabel et al., 1992).

Thus, T-cell mediated autoimmunity seems to underlie giant cell arteritis and Takayasu arteritis, although the relevant autoantigen(s) and epitopes, whether or not constitutive or modified proteins, are not identified. The disruption of vascular structures and expression of growth factors like platelet-derived growth factor (PDGF)-A and -B, and tumour growth factor (TGF)-β by the inflammatory infiltrate will lead to aneurysmatic dilatation and migration and proliferation of myofibroblasts in the intima and media with subsequent fibrosis and stenosis.

In other forms of primary vasculitis, T cells may be involved, but how and to what extent is unclear. In Kawasaki disease the infiltrate in small and medium-sized vessels is predominantly mononuclear. Some have suggested an association of this disease with certain HLA class II antigens, but this could not be confirmed by others. Likewise, data compatible with massive, antigen unrestricted, T-cell stimulation by superantigens from *Staphylococcus* spp. and *Streptococcus* spp. have been reported in this disease, but others failed to confirm this association. Again, the Th17 axis appears to be involved since increased proportions of Th17 cells in conjunction with decreased proportions of Treg cells has been reported (Jia et al., 2010). In polyarteritis nodosa, the inflammatory vascular infiltrate predominantly consists of $CD8^+$ T cells and macrophages suggesting cell-mediated immune mechanism in the pathogenesis of this disease (Panegyres et al., 1990). The precise role of these cells and the antigen or antigens against which these cells are directed are not known.

General discussion

Despite clear advances made in the last decades, the pathogenesis of vasculitis is not fully understood. General patterns of involvement of both the innate and adaptive immune system have been found and allow categorizing of the inflammatory response of the vasculitic syndromes as primarily immune complex mediated, autoantibody mediated, or T-cell mediated (Table 158.5). From the pathophysiology of the different syndromes discussed above it is clear that this pathophysiological distinction in three groups is a gross oversimplification. In many forms of vasculitis different types of immune responses are concurrently or sequentially operative. The proof that autoantibodies or autoreactive T cells are definitely the cause of vasculitis is for most vasculitic diseases

Table 158.5 Immune response pathways presumed to be involved in the pathogenesis of primary systemic vasculitis

Vasculitic syndrome	Immune complexes	Autoantibodies	(Auto-)reactive T-cells	Known autoantigens	Complement
Giant cell arteritis	–	–	+	–	–
Takayasu arteritis	–	+/–	+	–	–
Polyarteritis nodosa	+[a]	–	+/–	–	–
Kawasaki syndrome	–	+/–	+/–	–	–
Microscopic polyangiitis	–	++	+/–	+	±
Granulomatosis with polyangiitis	–	++	+/–	+	±
Churg–Strauss syndrome	–	+	–	–	–
Henoch–Schönlein purpura	++	–	–	–	+/–
Essential cryoglobulinaemic vasculitis	++	–	–	–	+
Cutaneous leucocytoclastic angiitis	++	–	–	–	+

– = not involved in pathogenesis; +/– = potentially involved, but maybe only in effector phase; + = likely to be involved; ++ = involved in pathogenesis.

[a] In hepatitis B-associated case.

absent. Furthermore, the antigens to which the autoreactive B- or T-cell response is directed are either not characterized or are tissue non-specific structures that are ubiquitously present. As vasculitic diseases are infrequent, it is clear that very specific circumstances have to be met with respect to the antigens, modified or not, and the response to these antigens. Likewise, the seemingly more elucidated pathogenesis of immune complex vasculitis is not understood, as the factors resulting in excessive immune complex formation and especially the fact that only a minority of these complexes result in vasculitis is concerned. How this occurs in a given patient at a given time is currently unclear. Models using inbred or genetically manipulated animals have provided proof of concept for some pathophysiological hypotheses like the role of cytotoxic T-cells in granulomatous large vessel vasculitis, immune complex mediated vasculitis, and ANCA-related vasculitis.

References

Abdulahad, W. H., Boots, A. M., and Kallenberg, C. G. (2011). FoxP3+ CD4+ T cells in systemic autoimmune diseases: the delicate balance between true regulatory T cells and effector Th-17 cells. *Rheumatology (Oxford)*, 50(4), 646–56.

Abdulahad, W. H., Kallenberg, C. G., Limburg, P. C., *et al.* (2009). Urinary CD4+ effector memory T cells reflect renal disease activity in antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum*, 60(9), 2830–8.

Abdulahad, W. H., Stegeman, C. A., and Kallenberg, C. G. (2009). Review article: The role of CD4(+) T cells in ANCA-associated systemic vasculitis. *Nephrology (Carlton)*, 14(1), 26–32.

Abdulahad, W. H., Stegeman, C. A., Limburg, P. C., *et al.* (2008). Skewed distribution of Th17 lymphocytes in patients with Wegener's granulomatosis in remission. *Arthritis Rheum*, 58(7), 2196–205.

Abdulahad, W. H., Stegeman, C. A., van der Geld, Y. M., *et al.* (2007). Functional defect of circulating regulatory CD4+ T cells in patients with Wegener's granulomatosis in remission. *Arthritis Rheum*, 56(6), 2080–91.

Abdulahad, W. H., van der Geld, Y. M., Stegeman, C. A., *et al.* (2006). Persistent expansion of CD4+ effector memory T cells in Wegener's granulomatosis. *Kidney Int*, 70(5), 938–47.

Ball, G. V. and Bridges, S. L., Jr. (2002). Pathogenesis of vasculitis. In G. V. Ball and S. L. Bridges, Jr. (eds.) *Vasculitis*, pp. 34–52. Oxford: Oxford University Press.

Ballieux, B. E., Zondervan, K. T., Kievit, P., *et al.* (1994). Binding of proteinase 3 and myeloperoxidase to endothelial cells: ANCA-mediated endothelial damage through ADCC? *Clin Exp Immunol*, 97(1), 52–60.

Bansal, P. J. and Tobin, M. C. (2004). Neonatal microscopic polyangiitis secondary to transfer of maternal myeloperoxidase-antineutrophil cytoplasmic antibody resulting in neonatal pulmonary hemorrhage and renal involvement. *Ann Allergy Asthma Immunol*, 93(4), 398–401.

Belizna, C., Duijvestijn, A., Hamidou, M., *et al.* (2006). Antiendothelial cell antibodies in vasculitis and connective tissue disease. *Ann Rheumat Dis*, 65(12), 1545–50.

Berden, A. E., Kallenberg, C. G., Savage, C. O., *et al.* (2009). Cellular immunity in Wegener's granulomatosis: characterizing T lymphocytes. *Arthritis Rheum*, 60(6), 1578–87.

Boomsma, M. M., Stegeman, C. A., van der Leij, M. J., *et al.* (2000). Prediction of relapses in Wegener's granulomatosis by measurement of antineutrophil cytoplasmic antibody levels: a prospective study. *Arthritis Rheum*, 43(9), 2025–33.

Bordron, A., Dueymes, M., Levy, Y., *et al.* (1998). The binding of some human antiendothelial cell antibodies induces endothelial cell apoptosis. *J Clin Invest*, 101(10), 2029–35.

Brack, A., Geisler, A., Martinez-Taboada, V. M., *et al.* (1997). Giant cell vasculitis is a T cell-dependent disease. *Mol Med*, 3(8), 530–43.

Brentjens, J. R., O'Connell, D. W., Albini, B., *et al.* (1975). Experimental chronic serum sickness in rabbits that received daily multiple and high doses of antigen: a systemic disease. *Ann N Y Acad Sci*, 254, 603–13.

Brouwer, E., Huitema, M. G., Mulder, A. H., *et al.* (1994). Neutrophil activation in vitro and in vivo in Wegener's granulomatosis. *Kidney Int*, 45(4), 1120–31.

Brouwer, E., Stegeman, C. A., Huitema, M. G., *et al.* (1994). T cell reactivity to proteinase 3 and myeloperoxidase in patients with Wegener's granulomatosis (WG). *Clin Exp Immunol*, 98(3), 448–53.

Capraru, D., Muller, A., Csernok, E., *et al.* (2008). Expansion of circulating NKG2D+ effector memory T-cells and expression of NKG2D-ligand MIC in granulomaous lesions in Wegener's granulomatosis. *Clin Immunol*, 127(2), 144–50.

Carvalho, D., Savage, C. O., Isenberg, D., *et al.* (1999). IgG anti-endothelial cell autoantibodies from patients with systemic lupus erythematosus or systemic vasculitis stimulate the release of two endothelial cell-derived mediators, which enhance adhesion molecule expression and leukocyte adhesion in an autocrine manner. *Arthritis Rheum*, 42(4), 631–40.

Casselman, B. L., Kilgore, K. S., Miller, B. F., *et al.* (1995). Antibodies to neutrophil cytoplasmic antigens induce monocyte chemoattractant protein-1 secretion from human monocytes. *J Lab Clin Med*, 126(5), 495–502.

Chan, T. M., Frampton, G., Jayne, D. R., *et al.* (1993). Clinical significance of anti-endothelial cell antibodies in systemic vasculitis: a longitudinal study comparing anti-endothelial cell antibodies and anti-neutrophil cytoplasm antibodies. *Am J Kidney Dis*, 22(3), 387–92.

Chavele, K. M., Shukla, D., Keteepe-Arachi, T., *et al.* (2010). Regulation of myeloperoxidase-specific T cell responses during disease remission in antineutrophil cytoplasmic antibody-associated vasculitis: the role of Treg cells and tryptophan degradation. *Arthritis Rheum*, 62(5), 1539–48.

Christian, C. L. and Sergent, J. S. (1976). Vasculitis syndromes: clinical and experimental models. *Am J Med*, 61(3), 385–92.

Ciavatta, D. J., Yang, J., Preston, G. A., *et al.* (2010). Epigenetic basis for aberrant upregulation of autoantigen genes in humans with ANCA vasculitis. *J Clin Invest*, 120(9), 3209–19.

Claudy, A. (1998). Pathogenesis of leukocytoclastic vasculitis. *Eur J Dermatol*, 8(2), 75–9.

Cochrane, C. G. and Hawkins, D. (1968). Studies on circulating immune complexes. 3. Factors governing the ability of circulating complexes to localize in blood vessels. *J Exp Med*, 127(1), 137–54.

Csernok, E., Trabandt, A., Muller, A., *et al.* (1999). Cytokine profiles in Wegener's granulomatosis: predominance of type 1 (Th1) in the granulomatous inflammation. *Arthritis Rheum*, 42(4), 742–50.

Cui, Z., Zhao, M. H., Segelmark, M., *et al.* (2010). Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. *Kidney Int*, 78(6), 590–7.

Cunningham, M. A., Huang, X. R., Dowling, J. P., *et al.* (1999). Prominence of cell-mediated immunity effectors in 'pauci-immune' glomerulonephritis. *J Am Soc Nephrol*, 10(3), 499–506.

Dammacco, F., Sansonno, D., Piccoli, C., *et al.* (2001). The cryoglobulins: an overview. *Eur J Clin Invest*, 31(7), 628–38.

Davies, D. J., Moran, J. E., Niall, J. F., *et al.* (1982). Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *Br Med J (Clin Res Ed)*, 285(6342), 606.

Davin, J. C. and Weening, J. J. (2001). Henoch–Schonlein purpura nephritis: an update. *Eur J Pediatr*, 160(12), 689–95.

Del Papa, N., Guidali, L., Sironi, M., *et al.* (1996). Anti-endothelial cell IgG antibodies from patients with Wegener's granulomatosis bind to human endothelial cells in vitro and induce adhesion molecule expression and cytokine secretion. *Arthritis Rheum*, 39(5), 758–66.

Del Papa, N., Meroni, P. L., Barcellini, W., *et al.* (1992). Antibodies to endothelial cells in primary vasculitides mediate in vitro endothelial cytotoxicity in the presence of normal peripheral blood mononuclear cells. *Clin Immunol Immunopathol*, 63(3), 267–74.

Deng, J., Younge, B. R., Olshen, R. A., *et al.* (2010). Th17 and Th1 T-cell responses in giant cell arteritis. *Circulation*, 121(7), 906–15.

Dijstelbloem, H. M., Bijl, M., Fijnheer, R., *et al.* (2000). Fcgamma receptor polymorphisms in systemic lupus erythematosus: association with disease and in vivo clearance of immune complexes. *Arthritis Rheum*, 43(12), 2793–800.

Dijstelbloem, H. M., Scheepers, R. H., Oost, W. W., *et al.* (1999). Fcgamma receptor polymorphisms in Wegener's granulomatosis: risk factors for disease relapse. *Arthritis Rheum*, 42(9), 1823–7.

Dixon, F. J., Vazquez, J. J., Weigle, W. O., *et al.* (1958). Pathogenesis of serum sickness. *Arch Pathol*, 65(1), 18–28.

Dolman, K. M., Stegeman, C. A., van de Wiel, B. A., *et al.* (1993). Relevance of classic anti-neutrophil cytoplasmic autoantibody (C-ANCA)-mediated inhibition of proteinase 3-alpha 1-antitrypsin complexation to disease activity in Wegener's granulomatosis. *Clin Exp Immunol*, 93(3), 405–10.

Donald, K. J., Edwards, R. L., and McEvoy, J. D. (1976). An ultrastructural study of the pathogenesis of tissue injury in limited Wegener's granulomatosis. *Pathology*, 8(2), 161–9.

Edberg, J. C., Wainstein, E., Wu, J., *et al.* (1997). Analysis of FcgammaRII gene polymorphisms in Wegener's granulomatosis. *Exp Clin Immunogenet*, 14(3), 183–95.

Ewert, B. H., Jennette, J. C., and Falk, R. J. (1992). Anti-myeloperoxidase antibodies stimulate neutrophils to damage human endothelial cells. *Kidney Int*, 41(2), 375–83.

Falk, R. J., Gross, W. L., Guillevin, L., *et al.* (2011). Granulomatosis with polyangiitis (Wegener's): an alternative name for Wegener's granulomatosis. *J Am Soc Nephrol*, 22(4), 587–8.

Falk, R. J. and Jennette, J. C. (1988). Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N Engl J Med*, 318(25), 1651–7.

Falk, R. J. and Jennette, J. C. (2002). ANCA are pathogenic—oh yes they are! *J Am Soc Nephrol*, 13(7), 1977–9.

Falk, R. J., Terrell, R. S., Charles, L. A., *et al.* (1990). Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc Natl Acad Sci U S A*, 87(11), 4115–19.

Fauci, A. S., Haynes, B., and Katz, P. (1978). The spectrum of vasculitis: clinical, pathologic, immunologic and therapeutic considerations. *Ann Intern Med*, 89(5 Pt 1), 660–76.

Frampton, G., Jayne, D. R., Perry, G. J., *et al.* (1990). Autoantibodies to endothelial cells and neutrophil cytoplasmic antigens in systemic vasculitis. *Clin Exp Immunol*, 82(2), 227–32.

Gan, P. Y., Steinmetz, O. M., Tan, D. S., *et al.* (2010). Th17 cells promote autoimmune anti-myeloperoxidase glomerulonephritis. *J Am Soc Nephrol*, 21(6), 925–31.

Griffith, M. E., Coulthart, A., and Pusey, C. D. (1996). T cell responses to myeloperoxidase (MPO) and proteinase 3 (PR3) in patients with systemic vasculitis. *Clin Exp Immunol*, 103(2), 253–8.

Grunwald, M. H., Avinoach, I., Amichai, B., *et al.* (1997). Leukocytoclastic vasculitis—correlation between different histologic stages and direct immunofluorescence results. *Int J Dermatol*, 36(5), 349–52.

Hagen, E. C., Daha, M. R., Hermans, J., *et al.* (1998). Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. *Kidney Int*, 53(3), 743–53.

Hall, J. B., Wadham, B. M., Wood, C. J., *et al.* (1984). Vasculitis and glomerulonephritis: a subgroup with an antineutrophil cytoplasmic antibody. *Aust N Z J Med*, 14(3), 277–8.

Harper, L. and Savage, C. O. (2000). Pathogenesis of ANCA-associated systemic vasculitis. *J Pathol*, 190(3), 349–59.

Heeringa, P., Brouwer, E., Tervaert, J. W., *et al.* (1998). Animal models of anti-neutrophil cytoplasmic antibody associated vasculitis. *Kidney Int*, 53(2), 253–63.

Heeringa, P. and Little, M. A. (2011). In vivo approaches to investigate ANCA-associated vasculitis: lessons and limitations. *Arthritis Res Ther*, 13(1), 204.

Heeringa, P. and Tervaert, J. W. (2004). Pathophysiology of ANCA-associated vasculitides: are ANCA really pathogenic? *Kidney Int*, 65(5), 1564–7.

Henson, P. M. and Cochrane, C. G. (1971). Acute immune complex disease in rabbits. The role of complement and of a leukocyte-dependent release of vasoactive amines from platelets. *J Exp Med*, 133(3), 554–71.

Huugen, D., van Esch, A., Xiao, H., *et al.* (2007). Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. *Kidney Int*, 71(7), 646–54.

Huugen, D., Xiao, H., van Esch, A., *et al.* (2005). Aggravation of anti-myeloperoxidase antibody-induced glomerulonephritis by bacterial lipopolysaccharide: role of tumor necrosis factor-alpha. *Am J Pathol*, 167(1), 47–58.

Ihrcke, N. S. and Platt, J. L. (1996). Shedding of heparan sulfate proteoglycan by stimulated endothelial cells: evidence for proteolysis of cell-surface molecules. *J Cell Physiol*, 168(3), 625–37.

Jancar, S. and Sanchez Crespo, M. (2005). Immune complex-mediated tissue injury: a multistep paradigm. *Trends Immunol*, 26(1), 48–55.

Jenne, D. E., Frohlich, L., Hummel, A. M., *et al.* (1997). Cloning and functional expression of the murine homologue of proteinase 3: implications for the design of murine models of vasculitis. *FEBS Lett*, 408(2), 187–90.

Jennette, J. C. and Falk, R. J. (1997). Small-vessel vasculitis. *N Engl J Med*, 337(21), 1512–23.

Jennette, J. C., Falk, R. J., Andrassy, K., *et al.* (1994). Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum*, 37(2), 187–92.

Jennette, J. C., Xiao, H., and Falk, R. J. (2006). Pathogenesis of vascular inflammation by anti-neutrophil cytoplasmic antibodies. *J Am Soc Nephrol*, 17(5), 1235–42.

Jia, S., Li, C., Wang, G., *et al.* (2010). The T helper type 17/regulatory T cell imbalance in patients with acute Kawasaki disease. *Clin Exp Immunol*, 162(1), 131–7.

Kain, R., Exner, M., Brandes, R., *et al.* (2008). Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. *Nat Med*, 14(10), 1088–96.

Karasawa, R., Kurokawa, M. S., Yudoh, K., *et al.* (2010). Peroxiredoxin 2 is a novel autoantigen for anti-endothelial cell antibodies in systemic vasculitis. *Clin Exp Immunol*, 161(3), 459–70.

Kauffmann, R. H., Herrmann, W. A., Meijer, C. J., *et al.* (1980). Circulating and tissue-bound immune complexes in allergic vasculitis: relationship between immunoglobulin class and clinical features. *Clin Exp Immunol*, 41(3), 459–70.

Kessenbrock, K., Krumbholz, M., Schonermarck, U., *et al.* (2009). Netting neutrophils in autoimmune small-vessel vasculitis. *Nat Med*, 15(6), 623–5.

Kettritz, R., Schreiber, A., Luft, F. C., *et al.* (2001). Role of mitogen-activated protein kinases in activation of human neutrophils by antineutrophil cytoplasmic antibodies. *J Am Soc Nephrol*, 12(1), 37–46.

Kitching, A. R. and Holdsworth, S. R. (2011). The emergence of TH17 cells as effectors of renal injury. *J Am Soc Nephrol*, 22(2), 235–8.

Kocher, M., Edberg, J. C., Fleit, H. B., *et al.* (1998). Antineutrophil cytoplasmic antibodies preferentially engage Fc gammaRIIIb on human neutrophils. *J Immunol*, 161(12), 6909–14.

Leung, D. Y., Geha, R. S., Newburger, J. W., *et al.* (1986). Two monokines, interleukin 1 and tumor necrosis factor, render cultured vascular endothelial cells susceptible to lysis by antibodies circulating during Kawasaki syndrome. *J Exp Med*, 164(6), 1958–72.

Levine, S. M. and Hellmann, D. B. (2002). Giant cell arteritis. *Curr Opin Rheumatol*, 14(1), 3–10.

Ley, K., Laudanna, C., Cybulsky, M. I., *et al.* (2007). Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol*, 7(9), 678–89.

Li, X., Ptacek, T. S., Brown, E. E., *et al.* (2009). Fcgamma receptors: structure, function and role as genetic risk factors in SLE. *Genes Immun*, 10(5), 380–9.

Lidar, M., Lipschitz, N., Langevitz, P., *et al.* (2009). The infectious etiology of vasculitis. *Autoimmunity*, 42(5), 432–8.

Lindqvist, K. J. and Osterland, C. K. (1971). Human antibodies to vascular endothelium. *Clin Exp Immunol*, 9(6), 753–60.
Lindsey, N. J., Henderson, F. I., Malia, R., *et al.* (1994). Inhibition of prostacyclin release by endothelial binding anticardiolipin antibodies in thrombosis-prone patients with systemic lupus erythematosus and the antiphospholipid syndrome. *Br J Rheumatol*, 33(1), 20–6.
Little, M. A., Bhangal, G., Smyth, C. L., *et al.* (2006). Therapeutic effect of anti-TNF-{alpha} antibodies in an experimental model of anti-neutrophil cytoplasm antibody-associated systemic vasculitis. *J Am Soc Nephrol*, 17(1), 160–9.
Little, M. A., Smyth, C. L., Yadav, R., *et al.* (2005). Antineutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interactions in vivo. *Blood*, 106(6), 2050–8.
Lobatto, S., Daha, M. R., Breedveld, F. C., *et al.* (1988). Abnormal clearance of soluble aggregates of human immunoglobulin G in patients with systemic lupus erythematosus. *Clin Exp Immunol*, 72(1), 55–9.
Ludviksson, B. R., Sneller, M. C., Chua, K. S., *et al.* (1998). Active Wegener's granulomatosis is associated with HLA-DR+ CD4+ T cells exhibiting an unbalanced Th1-type T cell cytokine pattern: reversal with IL-10. *J Immunol*, 160(7), 3602–9.
Mayadas, T. N., Tsokos, G. C., and Tsuboi, N. (2009). Mechanisms of immune complex-mediated neutrophil recruitment and tissue injury. *Circulation*, 120(20), 2012–24.
McCluskey, R. T. and Fienberg, R. (1983). Vasculitis in primary vasculitides, granulomatoses, and connective tissue diseases. *Hum Pathol*, 14(4), 305–15.
Michalak, T. (1978). Immune complexes of hepatitis B surface antigen in the pathogenesis of periarteritis nodosa. A study of seven necropsy cases. *Am J Pathol*, 90(3), 619–32.
Miyakawa, Y., Yamada, A., Kosaka, K., *et al.* (1981). Defective immune-adherence (C3b) receptor on erythrocytes from patients with systemic lupus erythematosus. *Lancet*, 2(8245), 493–7.
Morgan, M. D., Day, C. J., Piper, K. P., *et al.* (2010). Patients with Wegener's granulomatosis demonstrate a relative deficiency and functional impairment of T-regulatory cells. *Immunology*, 130(1), 64–73.
Mulder, A. H., Heeringa, P., Brouwer, E., *et al.* (1994). Activation of granulocytes by anti-neutrophil cytoplasmic antibodies (ANCA): a Fc gamma RII-dependent process. *Clin Exp Immunol*, 98(2), 270–8.
Mulder, A. H., Stegeman, C. A., and Kallenberg, C. G. (1995). Activation of granulocytes by anti-neutrophil cytoplasmic antibodies (ANCA) in Wegener's granulomatosis: a predominant role for the IgG3 subclass of ANCA. *Clin Exp Immunol*, 101(2), 227–32.
Muller Kobold, A. C., Kallenberg, C. G., and Tervaert, J. W. (1998). Leucocyte membrane expression of proteinase 3 correlates with disease activity in patients with Wegener's granulomatosis. *Br J Rheumatol*, 37(8), 901–7.
Muller Kobold, A.C., van der Geld, Y.M., Limburg, P.C., *et al.* (1999). Pathophysiology of ANCA-associated glomerulonephritis. *Nephrol Dial Transplant*, 14(6), 1366–75.
Nabel, E. G., Plautz, G., and Nabel, G. J. (1992). Transduction of a foreign histocompatibility gene into the arterial wall induces vasculitis. *Proc Natl Acad Sci U S A*, 89(11), 5157–61.
Nogueira, E., Hamour, S., Sawant, D., *et al.* (2010). Serum IL-17 and IL-23 levels and autoantigen-specific Th17 cells are elevated in patients with ANCA-associated vasculitis. *Nephrol Dial Transplant*, 25(7), 2209–17.
Nolan, S. L., Kalia, N., Nash, G. B., *et al.* (2008). Mechanisms of ANCA-mediated leukocyte-endothelial cell interactions in vivo. *J Am Soc Nephrol*, 19(5), 973–84.
Novak, J., Julian, B. A., Tomana, M., *et al.* (2008). IgA glycosylation and IgA immune complexes in the pathogenesis of IgA nephropathy. *Semin Nephrol*, 28(1), 78–87.
Panegyres, P. K., Blumbergs, P. C., Leong, A. S., *et al.* (1990). Vasculitis of peripheral nerve and skeletal muscle: clinicopathological correlation and immunopathic mechanisms. *J Neurol Sci*, 100(1–2), 193–202.
Pendergraft, W. F., III, Preston, G. A., Shah, R. R., *et al.* (2004). Autoimmunity is triggered by cPR-3(105–201), a protein complementary to human autoantigen proteinase-3. *Nat Med*, 10(1), 72–9.
Pfister, H., Ollert, M., Frohlich, L. F., *et al.* (2004). Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. *Blood*, 104(5), 1411–18.
Polzer, K., Soleiman, A., Baum, W., *et al.* (2008). Selective p38MAPK isoform expression and activation in antineutrophil cytoplasmatic antibody-associated crescentic glomerulonephritis: role of p38MAPKalpha. *Ann Rheumat Dis*, 67(5), 602–8.
Popa, E. R., Franssen, C. F., Limburg, P. C., *et al.* (2002). In vitro cytokine production and proliferation of T cells from patients with anti-proteinase 3- and antimyeloperoxidase-associated vasculitis, in response to proteinase 3 and myeloperoxidase. *Arthritis Rheum*, 46(7), 1894–904.
Popa, E. R., Stegeman, C. A., Bos, N. A., *et al.* (1999). Differential B- and T-cell activation in Wegener's granulomatosis. *J Allergy Clin Immunol*, 103(5 Pt 1), 885–94.
Porges, A. J., Redecha, P. B., Kimberly, W. T., *et al.* (1994). Anti-neutrophil cytoplasmic antibodies engage and activate human neutrophils via Fc gamma RIIa. *J Immunol*, 153(3), 1271–80.
Preston, G. A. and Falk, R. J. (2011). Autoimmunity: does autoantigen complementarity underlie PR3-ANCA AAV? *Nat Rev Rheumatol*, 7(8), 439–41.
Primo, V. C., Marusic, S., Franklin, C. C., *et al.* (2010). Anti-PR3 immune responses induce segmental and necrotizing glomerulonephritis. *Clin Exp Immunol*, 159(3), 327–37.
Radford, D. J., Luu, N. T., Hewins, P., *et al.* (2001). Antineutrophil cytoplasmic antibodies stabilize adhesion and promote migration of flowing neutrophils on endothelial cells. *Arthritis Rheum*, 44(12), 2851–61.
Ralston, D. R., Marsh, C. B., Lowe, M. P., *et al.* (1997). Antineutrophil cytoplasmic antibodies induce monocyte IL-8 release. Role of surface proteinase-3, alpha1-antitrypsin, and Fcgamma receptors. *J Clin Invest* 100(6), 1416–24.
Rarok, A. A., Limburg, P. C., and Kallenberg, C. G. (2003). Neutrophil-activating potential of antineutrophil cytoplasm autoantibodies. *J Leukoc Biol*, 74(1), 3–15.
Rarok, A. A., Stegeman, C. A., Limburg, P. C., *et al.* (2002). Neutrophil membrane expression of proteinase 3 (PR3) is related to relapse in PR3-ANCA-associated vasculitis. *J Am Soc Nephrol*, 13(9), 2232–8.
Rastaldi, M. P., Ferrario, F., Tunesi, S., *et al.* (1996). Intraglomerular and interstitial leukocyte infiltration, adhesion molecules, and interleukin-1 alpha expression in 15 cases of antineutrophil cytoplasmic autoantibody-associated renal vasculitis. *Am J Kidney Dis*, 27(1), 48–57.
Reumaux, D., Vossebeld, P. J., Roos, D., *et al.* (1995). Effect of tumor necrosis factor-induced integrin activation on Fc gamma receptor II-mediated signal transduction: relevance for activation of neutrophils by anti-proteinase 3 or anti-myeloperoxidase antibodies. *Blood*, 86(8), 3189–95.
Savage, C. O., Pottinger, B. E., Gaskin, G., *et al.* (1991). Vascular damage in Wegener's granulomatosis and microscopic polyarteritis: presence of anti-endothelial cell antibodies and their relation to anti-neutrophil cytoplasm antibodies. *Clin Exp Immunol*, 85(1), 14–9.
Savage, C. O., Pottinger, B. E., Gaskin, G., *et al.* (1992). Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Am J Pathol*, 141(2), 335–42.
Savige, J., Davies, D., Falk, R. J., *et al.* (2000). Antineutrophil cytoplasmic antibodies and associated diseases: a review of the clinical and laboratory features. *Kidney Int*, 57(3), 846–62.
Schifferli, J. A., Ng, Y. C., and Peters, D. K. (1986). The role of complement and its receptor in the elimination of immune complexes. *N Engl J Med*, 315(8), 488–95.
Schreiber, A., Rolle, S., Peripelittchenko, L., *et al.* (2010). Phosphoinositol 3-kinase-gamma mediates antineutrophil cytoplasmic autoantibody-induced glomerulonephritis. *Kidney Int*, 77(2), 118–28.
Schreiber, A., Xiao, H., Jennette, J. C., *et al.* (2009). C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *J Am Soc Nephrol*, 20(2), 289–98.

Seko, Y. (2002). Takayasu's arteritis: pathogenesis. In G. S. Hoffman and C. M. Weyand (eds.) *Inflammatory Disease of Blood Vessels*, pp. 443–53. New York: Marcel Dekker Inc.

Stegeman, C. A., Tervaert, J. W., Huitema, M. G., *et al.* (1993). Serum markers of T cell activation in relapses of Wegener's granulomatosis. *Clin Exp Immunol.*, 91(3), 415–20.

Stegeman, C. A., Tervaert, J. W., Sluiter, W. J., *et al.* (1994). Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener granulomatosis. *Ann Intern Med*, 120(1), 12–7.

Suzuki, H., Moldoveanu, Z., Hall, S., *et al.* (2008). IgA1-secreting cell lines from patients with IgA nephropathy produce aberrantly glycosylated IgA1. *J Clin Invest*, 118(2), 629–39.

Sylvestre, D. L. and Ravetch, J. V. (1994). Fc receptors initiate the Arthus reaction: redefining the inflammatory cascade. *Science*, 265(5175), 1095–8.

Tadema, H., Kallenberg, C. G., Stegeman, C. A., *et al.* (2011). Reactivity against complementary proteinase-3 is not increased in patients with PR3-ANCA-associated vasculitis. *PLoS One*, 6(3), e17972.

Taekema-Roelvink, M. E., Kooten, C., Kooij, S. V., *et al.* (2001). Proteinase 3 enhances endothelial monocyte chemoattractant protein-1 production and induces increased adhesion of neutrophils to endothelial cells by upregulating intercellular cell adhesion molecule-1. *J Am Soc Nephrol*, 12(5), 932–40.

Tervaert, J. W., Huitema, M. G., Hene, R. J., *et al.* (1990). Prevention of relapses in Wegener's granulomatosis by treatment based on antineutrophil cytoplasmic antibody titre. *Lancet*, 336(8717), 709–11.

Tervaert, J. W., Limburg, P. C., Elema, J. D., *et al.* (1991). Detection of autoantibodies against myeloid lysosomal enzymes: a useful adjunct to classification of patients with biopsy-proven necrotizing arteritis. *Am J Med*, 91(1), 59–66.

Trepo, C. G., Zucherman, A. J., Bird, R. C., *et al.* (1974). The role of circulating hepatitis B antigen/antibody immune complexes in the pathogenesis of vascular and hepatic manifestations in polyarteritis nodosa. *J Clin Pathol*, 27(11), 863–8.

Tse, W. Y., Abadeh, S., Jefferis, R., *et al.* (2000). Neutrophil FcgammaRIIIb allelic polymorphism in anti-neutrophil cytoplasmic antibody (ANCA)-positive systemic vasculitis. *Clin Exp Immunol*, 119(3), 574–7.

Van der Veen, B. S., Chen, M., Muller, R., *et al.* (2011). Effects of p38 mitogen-activated protein kinase inhibition on anti-neutrophil cytoplasmic autoantibody pathogenicity in vitro and in vivo. *Ann Rheumat Dis*, 70(2), 356–65.

Van der Woude, F. J., Rasmussen, N., Lobatto, S., *et al.* (1985). Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet*, 1(8426), 425–9.

Van Timmeren, M. M., Chen, M. and Heeringa, P. (2009). Review article: Pathogenic role of complement activation in anti-neutrophil cytoplasmic auto-antibody-associated vasculitis. *Nephrology (Carlton)*, 14(1), 16–25.

Weyand, C. M. and Goronzy, J. J. (1999). Arterial wall injury in giant cell arteritis. *Arthritis Rheum*, 42(5), 844–53.

Weyand, C. M., Tetzlaff, N., Bjornsson, J., *et al.* (1997). Disease patterns and tissue cytokine profiles in giant cell arteritis. *Arthritis Rheum*, 40(1), 19–26.

Weyand, C. M., Younge, B. R. and Goronzy, J. J. (2011). IFN-gamma and IL-17: the two faces of T-cell pathology in giant cell arteritis. *Curr Opin Rheumatol*, 23(1), 43–9.

Witko-Sarsat, V., Lesavre, P., Lopez, S., *et al.* (1999). A large subset of neutrophils expressing membrane proteinase 3 is a risk factor for vasculitis and rheumatoid arthritis. *J Am Soc Nephrol*, 10(6), 1224–33.

Xiao, H., Heeringa, P., Hu, P., *et al.* (2002). Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest*, 110(7), 955–63.

Xiao, H., Heeringa, P., Liu, Z., *et al.* (2005). The role of neutrophils in the induction of glomerulonephritis by anti-myeloperoxidase antibodies. *Am J Pathol*, 167(1), 39–45.

Xiao, H., Schreiber, A., Heeringa, P., *et al.* (2007). Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol*, 170(1), 52–64.

Xu, P. C., Cui, Z., Chen, M., *et al.* (2011). Comparison of characteristics of natural autoantibodies against myeloperoxidase and anti-myeloperoxidase autoantibodies from patients with microscopic polyangiitis. *Rheumatology (Oxford)*, 50(7), 1236–43.

Yang, J. J., Preston, G. A., Pendergraft, W. F., *et al.* (2001). Internalization of proteinase 3 is concomitant with endothelial cell apoptosis and internalization of myeloperoxidase with generation of intracellular oxidants. *Am J Pathol*, 158(2), 581–92.

CHAPTER 159

The patient with vasculitis: clinical aspects

Alan D. Salama

Overview

Primary systemic vasculitis is a term that encompasses a number of diverse immune-mediated clinical conditions, characterized by vessel inflammation, many of which can affect the kidney and lead to significant renal impairment. The most common cause of renal involvement is due to small vessel vasculitis, most of which is associated with circulating antineutrophil cytoplasmic antibody (ANCA) and features necrotizing inflammation of small blood vessels, within and outside the glomerulus. However, other vasculitides, involving medium- or large-sized vessels, such as polyarteritis nodosa (PAN), and Takayasu arteritis may also lead to renal involvement, through renal ischaemia.

The systemic vasculitides are idiopathic and thus differentiate them from other secondary forms of vasculitis, induced by drugs, infections, or malignancies, which in certain circumstances may also be associated with ANCA—leading to some diagnostic difficulties. Renal involvement from vasculitis has a significant impact on patient outcome, with increasing degrees of renal impairment being associated with greater mortality. Early diagnosis and timely treatment is therefore critical for improving patient outcomes. Patients often present due to extrarenal symptoms, while those with renal-limited forms of disease may present later with more established renal damage.

Small vessel vasculitis

Primary small vessel vasculitis affecting the kidney may result from four diverse clinical syndromes: granulomatosis with polyangiitis (GPA, formerly Wegener's granulomatosis), microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg–Strauss syndrome), or renal-limited vasculitis, which can be considered a form of MPA. These conditions have different patterns and frequencies of extrarenal involvement, but all can manifest as a pauci-immune focal segmental necrotizing glomerulonephritis that cannot be easily differentiated one from another histologically. In addition, anti-glomerular basement membrane (GBM) or Goodpasture disease is also a small vessel vasculitis, but will not be dealt with in this chapter.

It is important to note that secondary causes of vasculitis may exist—precipitated by infection (bacterial endocarditis, viral infections), malignancies, or drugs (such as cocaine or propylthiouracil). In some secondary cases, ANCA may also be detected, producing some diagnostic difficulty, but titres are often lower than in subjects with primary systemic vasculitides (Falk et al., 1990) or in an unusual pattern such as with specificity for both proteinase 3 (PR3) and myeloperoxidase (MPO) (Malle et al., 2000; Popa et al., 2002; Mansfield et al., 2011). It is therefore essential to exclude secondary causes, based on history and certain clinical features (Table 159.1) before initiating immunosuppression. Treatment of the aetiological factor in secondary cases can frequently lead to disease resolution without immunosuppression and with subsequent loss of ANCA positivity.

Classification

The vasculitides were originally classified according to criteria established by American College of Rheumatology (ACR) which considered seven different vasculitic syndromes, but excluded MPA (Fries et al., 1990). Subsequently, the Chapel Hill Consensus Criteria (CHCC) (Jennette et al., 1994) defined a nomenclature for the vasculitic syndromes including MPA and renal-limited vasculitis, mostly based on the size of vessel involved, but these were not intended for disease classification. More recently, the Chapel Hill Consensus has been updated (Jennette et al., 2012) using the new terminology, without eponyms for the small vessel diseases, incorporating ANCA, and new categories of disease such as variable vessel vasculitis (affecting any vessel size, such as Behçet disease). There have been other systems applied for classification of cohorts of ANCA-associated vasculitis (AAV) patients, which include the European Medicines Agency criteria (Watts et al., 2007). The European Medicines Agency criteria provided an algorithm for classification of all the small vessel vasculitides, including GPA, EGPA, and MPA.

A recent genome-wide association study (GWAS) has demonstrated a clear genetic differentiation between patients with PR3-ANCA and MPO-ANCA (Savige et al., 1999) (see 'Genetics'), which mirrors the known differences in clinical pattern that are found between patients with these two antibodies, suggesting that separating the conditions by clinical criteria may be less useful than considering the immunological response generated and genetic background, to better understand the underlying pathological processes and disease features. Indeed, with regards relapse, classifying patients based on ANCA specificity is a better predictor of flare compared to other methods of disease classification (Lionaki et al., 2012). The incorporation of ANCA specificity with other clinical features may be a preferred basis for classifying these conditions, rather than the traditional constellation of clinical signs or symptoms. This was the approach taken by the combined French and

Table 159.1 Features of secondary small vessel systemic vasculitis

Provoking factor	Condition	ANCA association	Key features	References
Infectious	Infective endocarditis, chronic viral hepatitis	Yes, common, may be dual positivity to PR3 and MPO	Hypocomplementaemia, bacterial cultures or viral serology	Falk et al., 1990; Popa et al., 2002; Mansfield et al., 2011
Malignancy	Lymphoma, solid organ malignancies	Recognized, but not frequent. ANCA positivity without evidence of vasculitis reported in certain haematological malignancies	Clinical history. Care as malignancies may often be diagnosed soon after ANCA vasculitis diagnosis and may not be clearly causally related	Falk et al., 1990; Shaw et al., 1992; Dhaygude et al., 2004
Drugs	Propylthiouracil carbimazole, cocaine	Yes, frequent, dual positivity to PR3 and MPO	Drug history, drug screening	Schlesier et al., 1995; Malle et al., 2000; Voth et al., 2009
Autoimmune diseases	Rheumatoid arthritis, systemic lupus erythematosus	Yes, unusual	Autoantibody screen, complement levels	Kerkar et al., 2011

European vasculitis study groups who suggested a new subclassification of patients with GPA or MPA, based on phenotypes which incorporated ANCA (Mahr et al., 2012). These authors used cluster analysis to define five different subgroups of patients which had disparate outcomes and relapse rates, placing patients into one of the following categories: renal AAV with PR3-ANCA, renal AAV without PR3-ANCA, non-renal AAV, cardiovascular AAV, and gastrointestinal AAV. In future, consideration of ANCA subtypes in addition to the clinical phenotype seems highly appropriate to best define the patient's disease course.

Critically, similar clinical features may occur in the absence of ANCA, such as cases of ANCA-negative EGPA (in almost 40% of cases), GPA (in 30% of limited disease), or renal-limited vasculitis which makes up almost 10% of patient cohorts presenting with renal disease; although these patients have not been assessed with regard to genetic susceptibility, in the same way as the ANCA-positive cohorts, they are generally considered within the AAV and managed in a similar manner to their ANCA positive counterparts. In EGPA, there appear to be phenotypic differences in those with or without ANCA, the former presenting with renal disease and polyneuropathy more often, while the latter may present more commonly with cardiovascular involvement (Morgan et al., 2011). Further work is needed to understand the basis behind these phenotypic variations and large cohorts are being assembled through various consortia to initiate genotype-phenotype analyses in different clinical groups.

Epidemiology

Small vessel AAV is commonest in adult, white patients, with a mean age of 60–75 from most series (Ntatsaki et al., 2010), with no significant female preponderance. Increasingly it is recognized in very elderly (> 80 years old) patients in whom it can account for almost 20% of cases biopsied for acute kidney injury (Moutzouris et al., 2009). Overall incidence and prevalence appears to vary depending on the clinical syndrome and the country of origin (Table 159.2), but combined incidence of 15–20 cases/million/year and prevalence of 100–400 cases/million are reported. Clearly, percentages of different syndromes in these series will also depend on how the conditions were classified. Geographical variations are reported in clinical phenotype and immunological reactivity, with GPA predominating in Northern Europe and MPA commoner in Southern Europe. In Japan, although the overall incidence of AAV is similar to that found in parts of the United Kingdom (Fujimoto et al., 2011), MPA predominates (constituting 83% of AAV) while many patients with GPA are found to have anti-MPO antibodies demonstrating a different epidemiological pattern to that found in Europe and Northern America. Similar findings are reported from Chinese cohorts (Chen et al., 2010). Additionally, demographic differences were found between Japanese and British cases with the latter being younger at presentation (Fujimoto et al., 2011). Data from one French urban series, found that patients with non-European ancestry, mainly from North Africa, made up 14–38% of patients with GPA, MPA, and EGPA, although this was a small cohort of only 45 patients with AAV (Mahr et al., 2004). Although reported in Afro-Caribbean patients, the overall incidence is low, with a prevalence of 4.1% of a cohort of 465 patients from a large London teaching hospital (A. Tanna et al., unpublished data) and with a slightly higher prevalence in an equal sized US-based cohort in which African American patients made up 13% of a cohort of 449 AAV collected by the Glomerular Disease Collaborative Network in North America (Cao et al., 2011). European white people are reported to have almost double the prevalence compared to New Zealand Maoris or New Zealander Asians (O'Donnell et al., 2007). Other ethnic susceptibility exists, with patients from the Indian subcontinent making up 13% of a large London cohort (A. Tanna et al., unpublished data), while there are significant cohorts of AAV patients reported from China (Chen et al., 2010) and India (Shankarkumar et al., 2005).

Table 159.2 Incidence and variation of disease subtype reported from around the world

Annual incidence per million population	UK	USA	Spain	Sweden	Norway	Greece	Japan
Granulomatosis with polyangiitis	10.8	8.6	4.8	9.8	8	6.6	0
Microscopic polyangiitis	5.1	2.9	7.9	2.5	2.7	10.2	14.8

Genetics

Genetic predisposition has long been suspected in AAV based on infrequent cases of familial disease, and following results of genetic polymorphisms found in small cohorts of patients. These polymorphic traits suggested that disease susceptibility was due to a number of common variants, each contributing a small amount to the overall susceptibility. Numerous immune gene variations have been implicated in susceptibility, as they are found in patients and not in control subjects, these include particular human leucocyte antigen (HLA) complex alleles, cytokines (such as interleukin (IL)-2, IL-10), other immune regulatory genes (such as CTLA4, Fc receptors), and anti-inflammatory genes (such as alpha 1 antitrypsin) (reviewed in Willcocks et al., 2010). In addition, the autoantigen PR3 has been implicated in cases of GPA, and its membrane-bound form was found to be upregulated in patients with GPA, and genetically regulated (Schreiber et al., 2003; von Vietinghoff et al., 2006).

Most recently a large European consortium conducted a GWAS which analysed > 1200 white, British patients with GPA or MPA compared with a matched healthy control population, and verified their findings from this discovery cohort in a validation cohort of over 1400 white, Northern European patients and similar numbers of controls (Abdulahad et al., 2009). GPA and MPA appeared to be genetically distinct, with separate HLA and non-HLA associated genetic risk factors. GPA was associated with HLA-DP, PR3, and alpha-1 antitrypsin, while MPA was associated with HLA-DQ; since there were many more GPA patients there may be other genetic risk factors for MPA which were not found due to lack of statistical power and further cases need to be analysed. Some of the previously reported associations from cohort studies did not reach levels of significance for GWAS and were therefore not validated in this study (Abdulahad et al., 2009).

Renal pathology

AAV leads to a pauci-immune, focal necrotizing glomerulonephritis (FNGN) with crescents (Fig. 159.1) and in a proportion of cases a marked tubulointerstitial nephritis. Less commonly a necrotizing arteritis is also seen, in 10–30% of cases (Stassen et al., 2008). Peri-glomerular accumulation of macrophages, neutrophils, and lymphocytes is common, and these peri-glomerular granulomas are found in all forms of AAV, while true interstitial granuloma are significantly rarer, but are almost uniquely found in GPA (Stassen et al., 2008). Clinical syndromes cannot be distinguished based on the renal biopsy findings since all three syndromes share a similar histological pattern, although EGPA biopsies may contain a predominant eosinophilic infiltrate. The degree of sclerosis and chronic damage may be greater in patients with MPA or renal-limited disease, and this is reflected in the presenting serum creatinine and long-term renal outcome (Tomasson et al., 2012). Pauci-immune FNGN is one of the most common diagnoses in patients presenting with acute kidney injury (Fig. 159.2), especially in the elderly, where it constituted just under 20% of findings in patients older than 80 years, who underwent renal biopsy (Moutzouris et al., 2009). In patients enrolled in European Vasculitis Study Group (EUVAS) trials, renal outcomes appeared to depend on the severity of the glomerular lesions and the degree of glomerular sclerosis found on renal biopsy (Berden et al., 2010), with the best outcome in those who had predominant focal necrotizing lesions and the worst in those with mainly sclerotic lesions, and this formed the basis of a new histological classification system (Table 159.3).

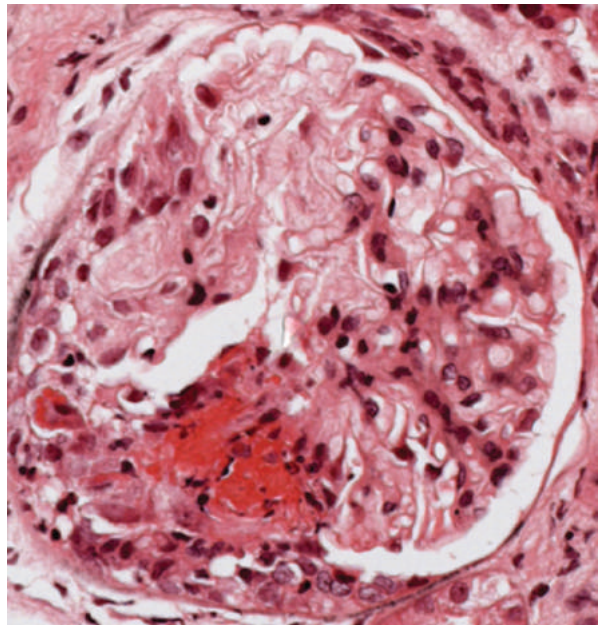

Fig. 159.1 Photomicrograph of a single glomerulus from a patient with AAV demonstrating segmental fibrinoid necrosis and thrombosis, tuft disruption, and an early cellular crescent (×400; haematoxylin and eosin).

Attempts to replicate these original findings in patients outside the context of clinical trials have mostly led to agreement with regard to the worst and best outcomes, found in sclerotic and focal classes respectively, but demonstrated variations in outcome in those with crescentic or mixed classes, suggesting that the mixed class may be too heterogenous or that other factors related to treatment may be critical in determining outcome (Chang et al., 2012).

Clinical features

The clinical features of the small vessel vasculitides vary depending on the constellation of organs involves, and this presentation may be highly variable, creating a diagnostic challenge. However, systemic constitutional features are frequent and may have been present for weeks or months prior to diagnosis, unlike anti-GBM disease. Constitutional symptoms include malaise, lethargy, fever, myalgia, arthralgia, anorexia, and in some cases marked weight loss. In recent clinical trials, between 56% and 66% of patients had such constitutional symptoms at presentation (Stone et al., 2010), while by contrast, skin involvement, most commonly with a purpuric rash or skin ulceration, was only found in 16–30% of patients (Stone et al., 2010; De Sousa et al., 2012) and mucous membrane or ocular involvement (Fig. 159.3) in 26% (Table 159.4). At presentation there is generally more than one organ system involved (Jones et al., 2010) and although any system can be involved, commonly these include the renal, respiratory (Fig. 159.4), neurological, and ear, nose, and throat (ENT) systems, with abdominal and cardiovascular involvement being generally less frequent (Stone et al., 2010). Pulmonary involvement is frequent and varied in presentation with pulmonary haemorrhage (Pepper et al., 2013), pulmonary fibrosis (Arulkumaran et al., 2011), granulomatous lung nodules (Travis et al., 1991), or asthma. The exact proportion of patients presenting with particular organ involvement in reported cohorts, will be influenced by geographical location (see 'Epidemiology'), the specialist service to which the patient was referred and the

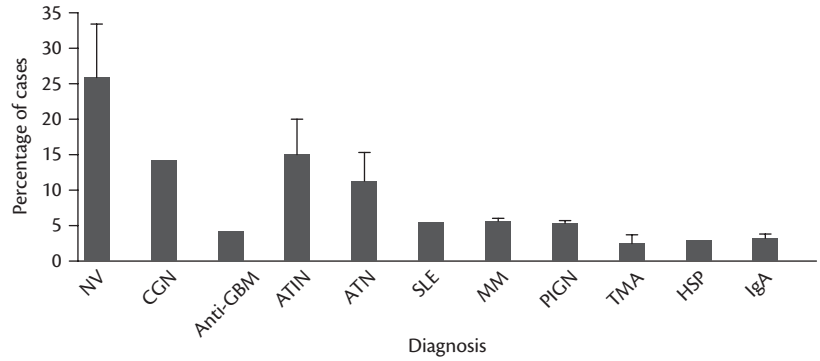

Fig. 159.2 Graph demonstrating the incidence of different renal pathologies in renal biopsies from adults taken for acute kidney injury.
NV: necrotizing nephritis; CGN: crescentic glomerulonephritis; anti-GBM: anti-glomerular basement membrane glomerulonephritis; ATIN: acute tubulo-interstitial nephritis; ATN: acute tubular necrosis; SLE: systemic lupus erythematosus; MM: multiple myeloma; PIGN: post infectious glomerulonephritis; TMA: thrombotic microangiopathy; HSP: Henoch-Schönlein purpura; IgA: IgA nephropathy.
Adapted from Schena et al. (1997) and Haas et al. (2000).

precise clinical syndrome they have. For example, cardiac disease is significantly more common in ANCA-negative EGPA than in either MPA or GPA (Stone et al., 2010; Morgan et al., 2011), while ENT involvement is more frequent in Northern European patients, who commonly present with GPA. Combined data from a number of EUVAS trials and European cohorts demonstrate the almost ubiquitous renal involvement in MPA, the predominant ENT and respiratory tract involvement in GPA, and the common neurological involvement in EGPA (Fig. 159.5). Overall the kidney remains the commonest organ affected and the most important with regard to patient outcomes, which is influenced by the degree of renal impairment at presentation and its response to therapy. Without extrarenal involvement, diagnosis of AAV may be delayed, and at the time of diagnosis patients may already have advanced renal impairment. The combination of renal impairment and pulmonary haemorrhage occurs in 25% of cases of AAV and is one of the commoner causes of pulmonary-renal syndrome (Fig. 159.6). The majority of patients with pulmonary haemorrhage have accompanying rapidly progressive glomerulonephritis, and the mortality in this group is extremely high (Pepper et al., 2013). Venous thromboembolic disease occurs in a proportion of patients at presentation related to the systemic inflammation (Mistry et al., 1996; Merkel et al., 2005; Stassen et al., 2008), and in some cases may coexist with haemorrhagic features (found in 20% of patients with pulmonary haemorrhage) (De Sousa et al., 2012), complicating disease management.

Table 159.3 Berden-EUVAS renal biopsy classification of ANCA-associated glomerulonephritis

Class	Diagnostic criteria (according to)
Focal	≥ 50% normal glomeruli
Crescentic	≥ 50% glomeruli containing cellular crescents
Sclerotic	≥ 50% glomeruli with global sclerosis
Mixed	< 50% glomeruli normal, < 50% crescentic, < 50% sclerotic

Required for inclusion in any one of these four classes, ≥ 1 glomerulus with necrotizing or crescentic glomerulonephritis on light microscopy and a pauci-immune staining pattern on immunofluorescence.
Adapted from Berden et al. (2010).

In some cases, medium and larger vessel involvement may occur with AAV and such overlap results in a wider array of clinical manifestations, such as prominent abdominal pain in patients who have overlapping medium vessel involvement (such as PAN) or headaches and polymyalgia symptoms in those with large vessel involvement (such as in giant cell arteritis). However, the disease is generally classified according to the smallest vessel involved.

Disease relapses are common (in 38% of > 500 patients enrolled in EUVAS trials) and occur more commonly in PR3-ANCA associated disease, in those with cardiovascular disease, and less in those with presenting creatinine of > 200 μmol/L (Walsh et al., 2012). Predicting relapses in individuals is difficult, with rising ANCA titres conferring only a likelihood risk of 2.8 (Tomasson et al., 2012). However, a particular CD8 T-cell signature appears to be able to separate populations at risk (McKinney et al., 2010), while other biomarkers such as serum calprotectin levels may also predict

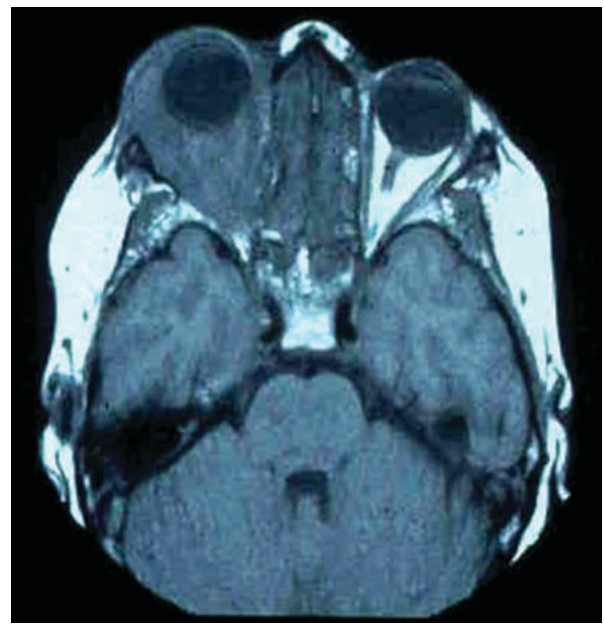

Fig. 159.3 CT of the orbits in a patient with granulomatosis with polyangiitis demonstrating extraocular granulomatous infiltration on the right (left hand side of the illustration) which is encasing and compressing the optic nerve.

Table 159.4 Clinical features in ANCA-associated vasculitis

Organ system	Symptoms and signs
General	Fever, myalgia, arthralgia
Skin/mucous membranes	Purpura, ulceration, infarct, gangrene, mouth or genital ulcers
Eye	Proptosis, episcleritis, scleritis, uveitis, blepharitis, keratitis, conjunctivitis
ENT	Epistaxis, nasal crusts, nasal polyps, nasal collapse, sinusitis, stridor, hoarseness, tracheal stenosis, hearing loss, otitis media. Rarely: aural chondritis
Respiratory	Breathlessness, cough, wheeze, haemoptysis, pulmonary nodules, pleural effusions, pleuritis, pulmonary fibrosis
Cardiovascular	Ischaemic chest pain, pericarditis, cardiomyopathy, valvular disease
Renal	Renal impairment, haematuria (macro- or microscopic) and proteinuria. Rarely: nephrotic syndrome, interstitial nephritis without glomerulonephritis
Neurological	Headaches, mononeuritis multiplex, peripheral neuropathy, cerebrovascular disease, meningitis, seizures, confusion
Abdominal	Pain, bloody diarrhoea, weight loss, peritonitis

relapse in early systemic disease (Pepper et al., 2013), but all these biomarker studies need to be replicated and tested prospectively in other populations before they can be recommended for clinical use.

Investigations

Making a diagnosis of AAV requires a combination of clinical, serological, radiological, and histological features. Depending on the combination of these, one may have lesser or greater diagnostic confidence. A high index of suspicion is often required to make a diagnosis of AAV as early clinical features may be non-specific and may mimic numerous infectious or inflammatory diseases. Careful history taking with emphasis on other possible vasculitic symptoms (Table 159.4) is critical as patients may often not volunteer certain symptoms which they think unrelated or which have been longstanding.

Serological testing for ANCA is performed by immunofluorescence and confirmed by enzyme-linked immunosorbant assay (ELISA) which also defines the antigenic ANCA target, as PR3 or MPO (Fig. 159.7), which has significant implication for outcome and management. Other ANCA targets (such as elastin, lactoferin, and bactericidal/permeability-increasing protein) have generally not been associated with primary systemic vasculitis. Recommendations are that ANCA screening be performed by immunofluorescence and confirmed in those positive by ELISA (Savige et al., 1999). New ELISA techniques with captured or immobilized antigen demonstrate increased sensitivity and require further study as to whether these can provide greater information regarding disease pathogenesis or outcomes (Csernok et al., 2004). Recently the potential importance of immunoglobulin A (IgA) ANCA, found in 30% of GPA patients, in mediating upper airway manifestations has also been highlighted (Kelley et al., 2011). ANCA may precede clinical diagnosis of AAV (Olson et al., 2011) and a period of careful observation and follow-up may be required in some patients who have minor symptomatology to exclude development of AAV. Using affinity (PR3 or MPO antigen) purified immunoglobulin, ANCA positivity was found in healthy controls' sera (Cui et al., 2010). These ANCAs were of significantly lower affinity than those from AAV patients and were not positive by conventional assays. Such data suggest that there is some loss of tolerance to these self-antigens, but that below a particular threshold, this autoreactivity is of no consequence as it does not lead to disease. These findings are reminiscent of other autoantigens to which degrees of cellular and humoral immunity may be found in healthy individuals.

ANCA positivity by conventional ELISA in a patient with renal disease and urinary abnormalities has a 95% positive predictive value for diagnosing AAV. However, there can be an overreliance on serological testing at the expense of histological confirmation of disease. More widespread use of ANCA testing in patients with a lower pre-test probability of AAV can result in a mistaken belief that the underlying disease is AAV. Indeed, ANCA immunofluorescence or even PR-3 ANCA ELISA positivity may occur in patients with other vasculitis mimics or secondary vasculitis syndromes, such as infectious endocarditis or viral disease, that can also present with renal impairment—but can be distinguished on renal biopsy findings (Falk et al., 1990).

In addition, even in patients with AAV, renal deterioration is not always due to recurrent pauci-immune glomerulonephritis, but may rarely be due to a drug-related tubulointerstitial nephritis, necessitating a renal biopsy to confirm the diagnosis (Salama et al., 2008). Renal biopsy is also essential for the 5–10% of patients who

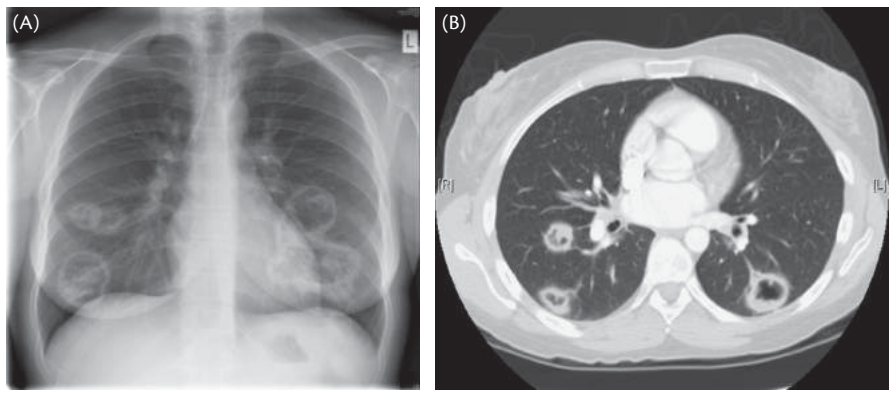

Fig. 159.4 (A) Chest radiograph and (B) CT of a patient with granulomatosis with polyangiitis demonstrating multiple cavitating lung nodules.

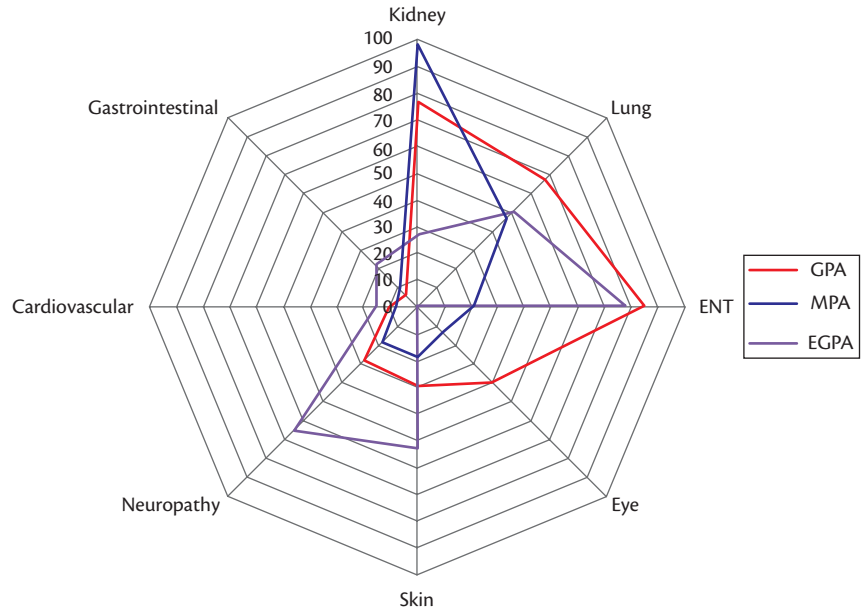

Fig. 159.5 Radar plot of the presenting features of patients with MPA, GPA, and EGPA from European Vasculitis Study Group and French Vasculitis Study Group cohorts. EGPA lung involvement excludes asthma, found in 95.7% of patients.
GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis; EGPA: eosinophilic granulomatosis with polyangiitis.
Adapted from Sinico et al. (2006) and Mahr et al. (2013).

are ANCA negative but have an indistinguishable disease with a pauci-immune focal necrotizing or crescentic glomerulonephritis as well as a proportion of patients who may have overlap syndromes (such as with anti-GBM disease, IgA glomerulonephritis, or scleroderma) in whom varied renal pathology may be expected and may inform management decisions. In those patients with predominant extrarenal disease, the finding of microscopic haematuria and/or proteinuria may prompt renal biopsy, and in many there will be evidence of a glomerulonephritis, however, in the absence of urinary abnormalities there is a low probability of finding renal vasculitis on biopsy. For a biopsy to confidently exclude a focal necrotizing process it has to be of sufficient size and for classification purposes a minimal sample size of 10 glomeruli was required, although 20 glomeruli provides a greater degree of diagnostic certainty that a focal process was not missed (Corwin et al., 1988). Sampling size and the focal nature of disease means that decisions to treat should not be made on the basis of the renal biopsy alone, as even with a sclerotic class, following treatment, 50% of patients had independent renal function at 5 years (Berden et al., 2010).

Other organs may yield positive biopsy findings to allow the diagnosis to be established (such as from lung nodules or nerve biopsies), however, biopsies from the retro-orbit, nasal mucosa,

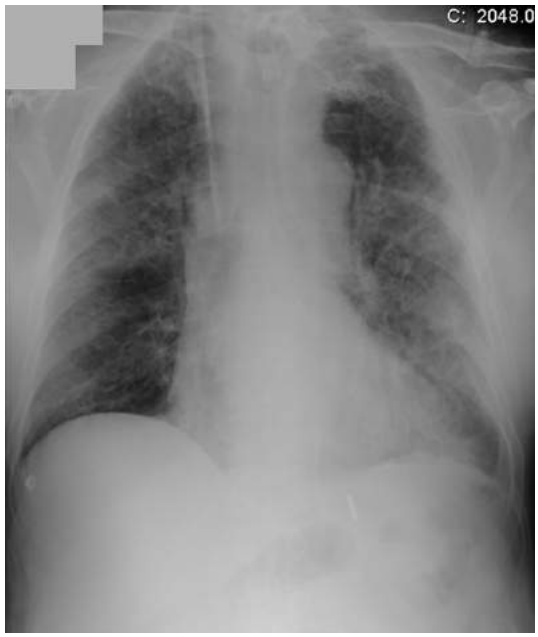

Fig. 159.6 Chest radiograph of a patient with MPO-ANCA associated vasculitis and pulmonary haemorrhage, demonstrating widespread intra-alveolar shadowing. Pulmonary haemorrhage may be confirmed by bronchoscopy or lung function tests where volume corrected gas transfer coefficient (KCO) is > 120% predicted.

Table 159.5 Clinical syndromes of ANCA-associated vasculitis

Syndrome	Key features
Granulomatosis with polyangiitis	Granulomatous inflammation of upper and lower respiratory tract. Sinusitis, nasal crusts, epistaxis, saddle nose deformity, pulmonary nodules, pulmonary haemorrhage, retro-orbital granuloma, subglottic stenosis. Renal involvement in 70–80% cases
Microscopic polyangiitis	Renal disease in 90–95% cases (100% of renal-limited vasculitis), pulmonary haemorrhage, pulmonary fibrosis, cutaneous and constitutional symptoms
Eosinophilic granulomatosis with polyangiitis	Asthma or nasal polyps Eosinophilia Myalgia, mononeuritis multiplex, cardiovascular involvement (40–50%), renal disease in 25% cases

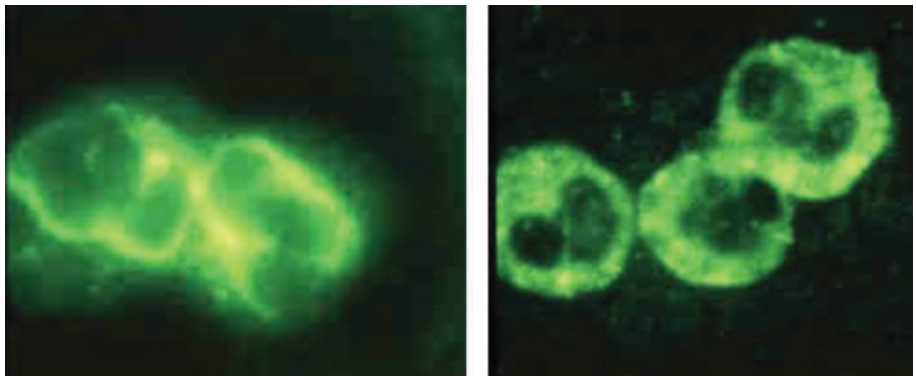

Fig. 159.7 Indirect immunofluorescence of ethanol fixed human neutrophils demonstrating positive ANCA staining with (A) perinuclear and (B) cytoplasmic staining patterns.

subglottis, or sinuses frequently only reveal chronic inflammatory changes.

Other organ involvement may require specific investigations to be carried out, such as nerve conduction studies (for mononeuritis multiplex or peripheral neuropathy), bronchoscopy, lung function tests, or high-resolution computed tomography (CT) scanning (for pulmonary fibrosis or haemorrhage).

Radiological features may aid in diagnosis and in certain circumstances may obviate the need for obtaining tissue for histology. For example, a patient with ENT symptoms, pulmonary cavitating nodules on chest X-ray or CT and a positive PR3-ANCA, a diagnosis of GPA may be made with reasonable confidence without pursuing a biopsy of the nodule, so long as other AAV mimics have been adequately excluded (such as tuberculosis).

Aetiology and pathogenesis

The strong clinical association between ANCA and GPA, MPA, and EGPA suggested that the autoantibody may play an important part in disease pathogenesis, despite the pauci-immune histological findings (see also Chapter 158). The evidence for ANCA pathogenicity in patients is less than in animal models of disease, however, overall the *in vitro* and *in vivo* findings are suggestive that ANCAs are more than biomarkers of disease and play an active role in initiating tissue damage. There remains much to be understood surrounding the role ANCA may play during disease remission, when titres may remain high in the absence of any clinical disease manifestations, or its role in the granulomatous manifestations of GPA, but ongoing genotype-phenotype research may provide significant advance in these areas.

Evidence for human ANCA pathogenicity comes from a single case report of a pregnant mother with relapsing AAV who transferred MPO-ANCA to her newborn (Bansal et al., 2004). However, other cases of MPO- or PR3-ANCA positivity and evidence of placental transfer, which did not lead to disease, have also been reported (Alfhaily et al., 2009; Silva et al., 2009).

ANCAs have been shown, *in vitro*, to induce cytokine-primed neutrophils to undergo a respiratory burst and degranulate (Falk et al., 1990; Keogan et al., 1992), release neutrophil extracellular traps (NETS) (Kessenbrock et al., 2009) and microparticles (Hong et al., 2012), induce endothelial activation and damage in neutrophil-endothelial co-cultures (Savage et al., 1992), and increase neutrophil adhesion to activated endothelium *in vitro* (Radford et al., 2001) and *in vivo* (Little et al., 2005; Nolan et al., 2008). Following cytokine priming of neutrophils, there is an upregulation of PR3 and MPO on the neutrophil cell surface, allowing greater ANCA binding and subsequent activation of the neutrophil, with further cytokine release and PR3/MPO upregulation. ANCAs also bind and activate neutrophils through Fc receptors, mediated through PI3K (Williams et al., 2003) and SyK (Hewins et al., 2004) signalling. Cytokine activation of the endothelium upregulates adhesion molecules and allows increased neutrophil adhesion and rolling along the endothelium, and following ANCA activation of the neutrophil, increased transmigration through the endothelial monolayer, with release of serine proteases and cytokines which in turn induce endothelial cell necrosis (Lu et al., 2006; Tull et al., 2012). Loss of normal endothelial integrity leads to activation of the complement and coagulation cascades with subsequent thrombosis. If this process occurs in the kidney, it leads to glomerular capillary thrombosis and necrosis, which may result in capillary wall rupture and release of the capillary contents into Bowman's space, initiating crescent formation.

The effect of ANCA in mediating disease *in vivo* has been shown in a murine model, in which anti-MPO antibodies were generated following MPO immunization of MPO-deficient mice, and subsequently passively transferred to naive wild type recipients, who developed a focal necrotizing crescentic glomerulonephritis and pulmonary capillaritis, resembling human disease (Xiao et al., 2002). This model is dependent on neutrophils and has been modified and refined by addition of lipopolysaccharide or priming with granulocyte-colony stimulating factor (Freeley et al., 2013), both manoeuvres leading to augmented disease, potentially through neutrophil recruitment and activation. Other modifications of this model, involve irradiation of the immunized MPO-deficient mice, which are then transplanted with wild-type bone marrow, to create chimeras with MPO-specific plasma cells making anti-MPO antibodies and leucocytes expressing MPO (Schreiber et al., 2006). A rat autoimmune model of MPO-ANCA vasculitis has also been generated, in which susceptible WKY rats are immunized with human MPO in adjuvant and develop anti-MPO antibodies, that cross react with rodent MPO-expressing leucocytes and induce a focal necrotizing crescentic glomerulonephritis with alveolar capillaritis (Little et al., 2009). Until recently, there was no convincing evidence for PR3-ANCA pathogenicity *in vivo*, as there was little disease induced following anti-PR3 antibody transfer, in a murine

model where anti-PR3 antibodies were induced in PR3/elastase deficient mice (Pfister et al., 2004). However, a single report of passive human PR3-ANCA transfer into humanized mice, immunodeficient mice which had been reconstituted with human stem cells, suggested that some mild glomerular abnormalities were produced, which were not found following transfer of control immunoglobulin (Little et al., 2011). These data require replication, but may now confirm pathogenicity of both MPO-and PR3-ANCA.

Cellular immunity is also implicated in AAV with T lymphocytes and monocytes/macrophages playing critical roles and showing evidence of persistent activation even during disease remission (Schlesier et al., 1995). T cells are found in the pathological lesions in AAV (within the kidney and within granulomata) and help in class switching of ANCA-producing B cells. Autoantigen-specific $CD4^+$ T cells are found in patients, reactive to PR3 or MPO (Griffith et al., 1996; Popa et al., 2002), with expansion of effector memory cells (Abdulahad et al., 2006), which migrate into tissues during disease flares and whose peripheral numbers inversely correlate with disease activity (Abdulahad et al., 2009). Moreover, an increased $CD4^+CD28^-$ T-cell population is found in the periphery and within granuloma which is related to chronic cytomegalovirus infection and immune exhaustion, with evidence that the proportion of these cells represents an independent risk factor for mortality (Morgan et al., 2011). In addition to the expanded effector compartment, there are abnormalities in regulatory T-cell (Treg) function, phenotype, or number that have been found in both PR3-ANCA- and MPO-ANCA-associated disease (Abdulahad et al., 2007; Chavele et al., 2010; Morgan et al., 2010), and linked with these defects are augmented Th17 cell populations with elevated IL-17 levels also implicated in disease pathogenesis (Abdulahad et al., 2008; Nogueira et al., 2010).

B-cell subsets may also influence disease activity apart from their role in ANCA production, with evidence that a regulatory B-cell population may exert immune suppressive effects, mediated in part through IL-10. The precise role of these B cell subsets in AAV remains to be clarified.

Although pauci-immune in nature, it has become apparent that complement plays an important role in the pathogenesis of AAV. This was first established in animal models, in which alternative pathway complement factor B or C5 deficiency was associated with attenuated disease, while blockade of the complement pathway with a C5 antagonist produced similar effects (Huugen et al., 2007; Xiao et al., 2007). More recently, analogous alternative pathway complement abnormalities have been reported in patients with AAV (Xing et al., 2009). For example, factor B, factor P, and C3d were found in renal biopsies from AAV patients, in association with the membrane attack complex C5-9, while elevated serum levels of C5a and its receptor C5L2 were measured in patients with active AAV (Pepper and Salama, 2012) and C5a signalling through its receptor was demonstrated to be critical in neutrophil priming of ANCA-mediated respiratory burst (Schreiber et al., 2009).

The mechanism of breakdown in immune tolerance that results in the generation of ANCAs remains unknown, but recent findings suggest that it may be related to abnormal macrophage mediated clearance of apoptotic PR3-expressing neutrophils (Kantari et al., 2007) or presentation of neutrophil autoantigens, contained within NETs (Fig. 159.8), to antigen-presenting cells and subsequent stimulation of T and B lymphocytes (Sangaletti et al., 2012). This failure to clear autoantigens, in combination with particular susceptibility genes, including different HLA alleles which constrain the antigenic peptide presented to T cells, may explain why ANCAs are generally directed at either PR3 or MPO but rarely both, outside the context of drug induced cases.

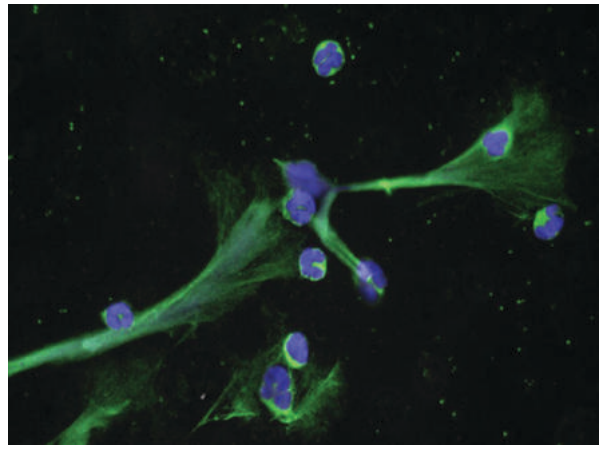

Fig. 159.8 Neutrophil extracellular traps stained for MPO (green) and nuclei (DAPI blue stain) demonstrating the chromatin fibres which contain significant quantities of MPO, which in turn may be taken up and presented by antigen presenting cells to T and B cells to initiate autoimmunity.

Environmental risk factors for disease

Genetic predisposition (see earlier) and environmental triggers are both necessary for disease initiation. There are many potential aetiological factors that may precede disease, but it is difficult to prove causality for many of these.

Infectious prodromes have long been recognized in AAV (Pinching et al., 1980), and more recently molecular mimicry between infectious agents and autoantigens have been proposed, with evidence for a high proportion of patients with FNGN having urinary tract infections with fimbriated bacteria in the months prior to presentation (Kain et al., 2008). The bacterial fimbrial protein, FimH, shares homology with an autoantigen, lysosomal membrane protein (LAMP)-2 present on neutrophils and glomerular endothelial cells, and a significant number of patients with FNGN have antibodies to this antigen at the time of presentation, linking bacterial reactivity to autoimmunity. Rodents immunized with FimH develop pauci-immune FNGN and antibodies to LAMP-2 (Kain et al., 2008), data which are reminiscent of earlier studies in which rats immunized with sonicated bacterial proteins developed ANCAs and evidence of glomerulonephritis (Savige et al., 2002). There has been some debate regarding the reproducibility and clinical significance of these anti-LAMP-2 antibodies, and further work is needed to confirm their precise role (Schebendach et al., 2012; Pepper et al., 2013). In addition, nasal carriage of *Staphylococcus aureus* has been associated with increased risk of disease activity and relapse (Stegeman et al., 1994; Laudien et al., 2010), while prophylactic antibacterial therapy appears to reduce relapse rates (Stegeman et al., 1996). CpG motifs found in bacterial DNA can stimulate patients' B cells to produce ANCAs *in vitro*, again linking infections with disease activation (Tadema et al., 2011a). Recently, others have proposed that following infection with for example *Staphylococcus aureus*, complementary PR3 antibodies are formed, which act as a template for anti-PR3 antibodies, through an anti-idiotypic network. They found increased proportions of

patients with anti-complementary PR3 antibodies compared to controls (21% in PR3-ANCA patients vs 1% in healthy and disease controls) (Pendergraft et al., 2004), but these data were not replicated in a separate European AAV cohort using similar techniques (Tadema et al., 2011b).

Other environmental factors have been associated with disease including drugs (such as propylthiouracil, contaminated cocaine and anti-tumour necrosis factor (TNF) biologics) (Schlesier et al., 1995; Malle et al., 2000; Saint Marcoux et al., 2006; Ashok et al., 2008; Arends et al., 2010; Hirohama et al., 2010) and prolonged exposure to silica dust (Beaudreuil et al., 2005; Hogan et al., 2007), although this later compound is widespread and causality is again hard to establish.

Medium and large vessel vasculitides and the kidney

Vessels other than the small glomerular vessels can be affected by necrotizing inflammation and lead to renal impairment, predominantly through ischaemic renal insults and renal hypertension; diagnosis is established radiologically and renal biopsy is generally not necessary.

Polyarteritis nodosa

PAN is significantly rarer than small vessel AAV, and is related to hepatitis B viral (HBV) infection in approximately a third of adult patients but few paediatric cases (Dillon et al., 2010) while other adult cases may be associated with other viral infections or malignancies.

Renal involvement in PAN is common, as a consequence of an ischaemic nephropathy, with > 66% of patients demonstrating renal artery aneurysms or stenoses (Fig. 159.9A) and a third of patients presenting with recent onset of hypertension in one large French cohort (Pagnoux et al., 2010). Although hypertension is generally mild, in 5% cases it may follow an accelerated phase and this is more common in HBV-associated PAN. Urinary abnormalities were not as frequent with 15–22% of patients presenting with haematuria or proteinuria (> 0.4 g/24 hours) respectively, while renal impairment (as defined by a serum creatinine >140 μmol/L) was only found in 15% of subjects, and mean presenting creatinine in this cohort of 348 patients was 112 μmol/L (Pagnoux et al., 2010). Advanced renal impairment requiring dialysis is less common, but well recognized and may be a consequence of renal infarction (Fig. 159.9B), more common in HBV-associated cases, or bilateral renal haemorrhage following rupture of renal microaneurysms (El Madhoun et al., 2009). Aneurysms may rupture spontaneously or following renal biopsy and can be treated with embolization or in some cases necessitate nephrectomy.

Diagnosis is based on finding elevated acute-phase reactants, erythrocyte sedimentation rate, C-reactive protein, and platelets, an absence of ANCAs, and positive angiographic evidence of microaneurysms and stenoses in medium-sized vessels.

Kawasaki disease

Kawasaki disease (KD) is a medium vessel vasculitis which usually occurs in children and is associated with a mucocutaneous lymph node syndrome. Larger vessels may also be involved, such as the aorta, but it is common to find coronary artery involvement in 15–25% of cases (Dillon et al., 2010). Renal dysfunction in KD may result from renal artery vasculitis, which frequently coincides with coronary artery involvement (Wang et al., 2007) and may be found in approximately 50% of subjects and rarely due to tubulointerstitial nephritis (Bonany et al., 2002). Six months following presentation 46% of patients were found to have evidence of renal scarring on DMSA scanning, which was more common in those patients with an abnormal renal ultrasound at presentation and demonstrated the long term renal impact of acute KD (Wang et al., 2007).

Takayasu arteritis

Takayasu arteritis (TA) is an idiopathic, chronic, granulomatous, large vessel vasculitis that principally affects the aorta, its branches, and the pulmonary artery. It may affect the renal vasculature with development of renal artery stenoses and result in renovascular hypertension and chronic kidney disease. In one cohort of Mexican patients, 47% had renal artery involvement with partial or complete occlusion (Soto et al., 2008), and between 33% and 73% patients had hypertension at presentation. In one autopsy series, higher levels of arterial wall inflammation were associated with a diffuse mesangial glomerulonephritis (de Pablo et al., 2007), while others have reported other glomerular lesions, such as glomerulosclerosis and mesangiocapillary glomerulonephritis (Li et al., 2009), in patients without renal artery involvement. Whether these really represent pathological associations or are coincidental findings is not clear.

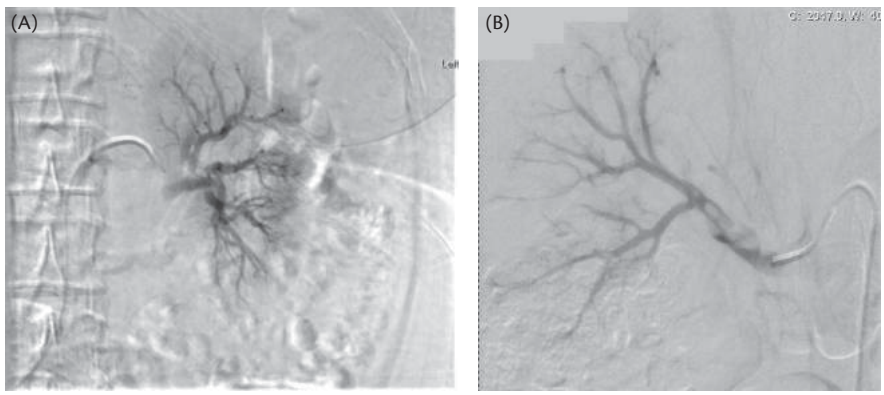

Fig. 159.9 Renal angiogram in two patients with polyarteritis nodosa demonstrating (A) numerous microaneurysms and (B) renal artery thrombus causing significant occlusion of the renal artery and additional evidence of tapering of the intrarenal vasculature.

References

Abdulahad, W. H., Kallenberg, C. G., Limburg, P. C., *et al.* (2009). Urinary CD4+ effector memory T cells reflect renal disease activity in antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum*, 60(9), 2830–8.

Abdulahad, W. H., Stegeman, C. A., Limburg, P. C., *et al.* (2008). Skewed distribution of Th17 lymphocytes in patients with Wegener's granulomatosis in remission. *Arthritis Rheum*, 58(7), 2196–205.

Abdulahad, W. H., Stegeman, C. A., van der Geld, Y. M., *et al.* (2007). Functional defect of circulating regulatory CD4+ T cells in patients with Wegener's granulomatosis in remission. *Arthritis Rheum*, 56(6), 2080–91.

Abdulahad, W. H., van der Geld, Y. M., Stegeman, C. A., *et al.* (2006). Persistent expansion of CD4+ effector memory T cells in Wegener's granulomatosis. *Kidney Int*, 70(5), 938–47.

Alfhaily, F., Watts, R., and Leather, A. (2009). Wegener's granulomatosis occurring de novo during pregnancy. *Clin Exp Rheumat*, 27(1 Suppl 52), S86–8.

Arends, S., Lebbink, H. R., Spoorenberg, A., *et al.* (2010). The formation of autoantibodies and antibodies to TNF-alpha blocking agents in relation to clinical response in patients with ankylosing spondylitis. *Clin Exp Rheumat*, 28(5), 661–8.

Arulkumaran, N., Periselneris, N., Gaskin, G., *et al.* (2011). Interstitial lung disease and ANCA-associated vasculitis: a retrospective observational cohort study. *Rheumatology (Oxford)*, 50(11), 2035–43.

Ashok, D., Dubey, S., and Tomlinson, I. (2008). C-ANCA positive systemic vasculitis in a patient with rheumatoid arthritis treated with infliximab. *Clin Rheumatol*, 27(2), 261–4.

Bansal, P. J. and Tobin, M. C. (2004). Neonatal microscopic polyangiitis secondary to transfer of maternal myeloperoxidase-antineutrophil cytoplasmic antibody resulting in neonatal pulmonary hemorrhage and renal involvement. *Ann Allergy Asthma Immunol*, 93(4), 398–401.

Beaudreuil, S., Lasfargues, G., Laueriere, L., *et al.* (2005). Occupational exposure in ANCA-positive patients: a case-control study. *Kidney Int*, 67(5), 1961–6.

Berden, A. E., Ferrario, F., Hagen, E. C., *et al.* (2010). Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol*, 21(10), 1628–36.

Bonany, P. J., Bilkis, M. D., Gallo, G., *et al.* (2002). Acute renal failure in typical Kawasaki disease. *Pediatr Nephrol*, 17(5), 329–31.

Cao, Y., Schmitz, J. L., Yang, J., *et al.* (2011). DRB1*15 allele is a risk factor for PR3-ANCA disease in African Americans. *J Am Soc Nephrol*, 22(6), 1161–7.

Chang, D. Y., Wu, L. H., Liu, G., *et al.* (2012). Re-evaluation of the histopathologic classification of ANCA-associated glomerulonephritis: a study of 121 patients in a single center. *Nephrology Dial Transplant*, 27(6), 2343–9.

Chavele, K. M., Shukla, D., Keteepe-Arachi, T., *et al.* (2010). Regulation of myeloperoxidase-specific T cell responses during disease remission in antineutrophil cytoplasmic antibody-associated vasculitis: the role of Treg cells and tryptophan degradation. *Arthritis Rheum*, 62(5), 1539–48.

Chen, M., Cui, Z., and Zhao, M. H. (2010). ANCA-associated vasculitis and anti-GBM disease: the experience in China. *Nephrol Dial Transplant*, 25(7), 2062–5.

Corwin, H. L., Schwartz, M. M., and Lewis, E. J. (1988). The importance of sample size in the interpretation of the renal biopsy. *Am J Nephrol*, 8(2), 85–9.

Csernok, E., Holle, J., Hellmich, B., *et al.* (2004). Evaluation of capture ELISA for detection of antineutrophil cytoplasmic antibodies directed against proteinase 3 in Wegener's granulomatosis: first results from a multicentre study. *Rheumatology (Oxford)*, 43(2), 174–80.

Cui, Z., Zhao, M. H., Segelmark, M., *et al.* (2010). Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. *Kidney Int*, 78(6), 590–7.

De Pablo, P., Garcia-Torres, R., Uribe, N., *et al.* (2007). Kidney involvement in Takayasu arteritis. *Clin Exp Rheumat*, 25(1 Suppl 44), S10–4.

De Sousa, E., Smith, R., Chaudhry, A., *et al.* (2012). Venous thromboembolism with concurrent pulmonary haemorrhage in systemic vasculitis. *Nephrol Dial Transplant*, 27(12), 4357–61.

Dhaygude, A., Griffith, M., Cairns, T., *et al.* (2004). Prolonged treatment with low-dose intravenous pulse cyclophosphamide may reduce rate of relapse in ANCA-associated vasculitis. *Nephron Clin Pract*, 97(4), 154–9.

Dillon, M. J., Eleftheriou, D., and Brogan, P. A. (2010). Medium-size-vessel vasculitis. *Pediatr Nephrol*, 25(9), 1641–52.

El Madhoun, I., Warnock, N. G., Roy, A., *et al.* (2009). Bilateral renal hemorrhage due to polyarteritis nodosa wrongly attributed to blunt trauma. *Nat Rev Urol*, 6(10), 563–7.

Falk, R. J., Hogan, S., Carey, T. S., *et al.* (1990). Clinical course of anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and systemic vasculitis. The Glomerular Disease Collaborative Network. *Ann Intern Med*, 113(9), 656–63.

Falk, R. J., Terrell, R. S., Charles, L. A., *et al.* (1990). Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc Natl Acad Sci U S A*, 87(11), 4115–19.

Freeley, S. J., Coughlan, A. M., Popat, R. J., *et al.* (2013). Granulocyte colony stimulating factor exacerbates antineutrophil cytoplasmic antibody vasculitis. *Ann Rheum Dis*, 72(6), 1053–8.

Fries, J. F., Hunder, G. G., Bloch, D. A., *et al.* (1990). The American College of Rheumatology 1990 criteria for the classification of vasculitis. Summary. *Arthritis Rheum*, 33(8), 1135–6.

Fujimoto, S., Watts, R. A., Kobayashi, S., *et al.* (2011). Comparison of the epidemiology of anti-neutrophil cytoplasmic antibody-associated vasculitis between Japan and the U.K. *Rheumatology (Oxford)*, 50(10), 1916–20.

Griffith, M. E., Coulthart, A., and Pusey, C. D. (1996). T cell responses to myeloperoxidase (MPO) and proteinase 3 (PR3) in patients with systemic vasculitis. *Clin Exp Immunol*, 103(2), 253–8.

Haas, M., Spargo, B. H., Wit, E. J., *et al.* (2000). Etiologies and outcome of acute renal insufficiency in older adults: a renal biopsy study of 259 cases. *Am J Kidney Dis*, 35(3), 433–47.

Hewins, P., Williams, J. M., Wakelam, M. J., *et al.* (2004). Activation of Syk in neutrophils by antineutrophil cytoplasm antibodies occurs via Fcgamma receptors and CD18. *J Am Soc Nephrol*, 15(3), 796–808.

Hirohama, D., Hoshino, J., Hasegawa, E., *et al.* (2010). Development of myeloperoxidase-antineutrophil cytoplasmic antibody-associated renal vasculitis in a patient receiving treatment with anti-tumor necrosis factor-alpha. *Mod Rheumatol*, 20(6), 602–5.

Hogan, S. L., Cooper, G. S., Savitz, D. A., *et al.* (2007). Association of silica exposure with anti-neutrophil cytoplasmic autoantibody small-vessel vasculitis: a population-based, case-control study. *Clin J Am Soc Nephrol*, 2(2), 290–9.

Hong, Y., Eleftheriou, D., Hussain, A. A., *et al.* (2012). Anti-neutrophil cytoplasmic antibodies stimulate release of neutrophil microparticles. *J Am Soc Nephrol*, 23(1), 49–62.

Huugen, D., van Esch, A., Xiao, H., *et al.* (2007). Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. *Kidney Int*, 71(7), 646–54.

Jennette, J. C., Falk, R. J., Andrassy, K., *et al.* (1994). Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum*, 37(2), 187–92.

Jennette, J. C., Falk, R. J., Bacon, P. A., *et al.* (2013). 2012 revised International Chapel Hill Consensus Conference nomenclature of vasculitides. *Arthritis Rheum*, 65(1), 1–11.

Jones, R. B., Tervaert, J. W., Hauser, T., *et al.* (2010). Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med*, 363(3), 211–20.

Kain, R., Exner, M., Brandes, R., *et al.* (2008). Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. *Nat Med*, 14(10), 1088–96.

Kantari, C., Pederzoli-Ribeil, M., Amir-Moazami, O., *et al.* (2007). Proteinase 3, the Wegener autoantigen, is externalized during neutrophil apoptosis: evidence for a functional association with phospholipid scramblase 1 and interference with macrophage phagocytosis. *Blood*, 110(12), 4086–95.

Kelley, J. M., Monach, P. A., Ji, C., *et al.* (2011). IgA and IgG antineutrophil cytoplasmic antibody engagement of Fc receptor genetic variants influences granulomatosis with polyangiitis. *Proc Natl Acad Sci U S A*, 108(51), 20736–41.

Keogan, M. T., Esnault, V. L., Green, A. J., *et al.* (1992). Activation of normal neutrophils by anti-neutrophil cytoplasm antibodies. *Clin Exp Immunol*, 90(2), 228–34.

Kerkar, S. P., Sanchez-Perez, L., Yang, S., *et al.* (2011). Genetic engineering of murine CD8+ and CD4+ T cells for preclinical adoptive immunotherapy studies. *J Immunother*, 34(4), 343–52.

Kessenbrock, K., Krumbholz, M., Schonermarck, U., *et al.* (2009). Netting neutrophils in autoimmune small-vessel vasculitis. *Nat Med*, 15(6), 623–5.

Laudien, M., Gadola, S. D., Podschun, R., *et al.* (2010). Nasal carriage of Staphylococcus aureus and endonasal activity in Wegener s granulomatosis as compared to rheumatoid arthritis and chronic Rhinosinusitis with nasal polyps. *Clin Exp Rheumat*, 28(1 Suppl 57), 51–5.

Li, X. M., Ye, W. L., Wen, Y. B., *et al.* (2009). Glomerular disease associated with Takayasu arteritis: 6 cases analysis and review of the literature. *Chin Med Sci J*, 24(2), 69–75.

Lionaki, S., Blyth, E. R., Hogan, S. L., *et al.* (2012). Classification of antineutrophil cytoplasmic autoantibody vasculitides: The role of antineutrophil cytoplasmic autoantibody specificity for myeloperoxidase or proteinase 3 in disease recognition and prognosis. *Arthritis Rheumat*, 64(10), 3452–62.

Little, M. A., Al-Ani, B., Ren, S., *et al.* (2011). Human Anti-PR3 ANCA recapitulate systemic vasculitis in mice with a humanised immune system. *Clin Exp Immunol*, 164 Suppl 1, 124.

Little, M. A., Smyth, C. L., Yadav, R., *et al.* (2005). Antineutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interactions in vivo. *Blood*, 106(6), 2050–8.

Little, M. A., Smyth, L., Salama, A. D., *et al.* (2009). Experimental Autoimmune Vasculitis: an animal model of ANCA-associated systemic vasculitis. *Am J Path*, 174, 1212–20.

Lu, X., Garfield, A., Rainger, G. E., *et al.* (2006). Mediation of endothelial cell damage by serine proteases, but not superoxide, released from antineutrophil cytoplasmic antibody-stimulated neutrophils. *Arthritis Rheum*, 54(5), 1619–28.

Mahr, A., Guillevin, L., Poissonnet, M., *et al.* (2004). Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis, and Churg-Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. *Arthritis Rheum*, 51(1), 92–9.

Mahr, A., Katsahian, S., Varet, H., *et al.* (2013). Revisiting the classification of clinical phenotypes of anti-neutrophil cytoplasmic antibody-associated vasculitis: a cluster analysis. *Ann Rheum Dis*, 72(6), 1003–10.

Malle, E., Waeg, G., Schreiber, R., *et al.* (2000). Immunohistochemical evidence for the myeloperoxidase/H2O2/halide system in human atherosclerotic lesions: colocalization of myeloperoxidase and hypochlorite-modified proteins. *Eur J Biochem*, 267(14), 4495–503.

Mansfield, N., Hamour, S., Habib, A. M., *et al.* (2011). Prolonged disease-free remission following rituximab and low-dose cyclophosphamide therapy for renal ANCA-associated vasculitis. *Nephrol Dial Transplant*, 26(10), 3280–6.

McKinney, E. F., Lyons, P. A., Carr, E. J., *et al.* (2010). A CD8+ T cell transcription signature predicts prognosis in autoimmune disease. *Nat Med*, 16(5), 586–91.

Merkel, P. A., Lo, G. H., Holbrook, J. T., *et al.* (2005). Brief communication: high incidence of venous thrombotic events among patients with Wegener granulomatosis: the Wegener's Clinical Occurrence of Thrombosis (WeCLOT) Study. *Ann Intern Med*, 142(8), 620–6.

Mistry, N., Pusey, C. D., and Gaskin, G. (1996). Venous thrombosis in systemic vasculitis. *Sarcoidosis Vasc Diffuse Lung Dis*, 13(3), 270.

Morgan, M. D., Day, C. J., Piper, K. P., *et al.* (2010). Patients with Wegener's granulomatosis demonstrate a relative deficiency and functional impairment of T-regulatory cells. *Immunology*, 130(1), 64–73.

Morgan, M. D., Pachnio, A., Begum, J., *et al.* (2011). CD4+CD28- T cell expansion in granulomatosis with polyangiitis (Wegener's) is driven by latent cytomegalovirus infection and is associated with an increased risk of infection and mortality. *Arthritis Rheum*, 63(7), 2127–37.

Moutzouris, D. A., Herlitz, L., Appel, G. B., *et al.* (2009). Renal biopsy in the very elderly. *Clin J Am Soc Nephrol*, 4(6), 1073–82.

Nogueira, E., Hamour, S., Sawant, D., *et al.* (2010). Serum IL-17 and IL-23 levels and autoantigen-specific Th17 cells are elevated in patients with ANCA-associated vasculitis. *Nephrol Dial Transplant*, 25(7), 2209–17.

Nolan, S. L., Kalia, N., Nash, G. B., *et al.* (2008). Mechanisms of ANCA-mediated leukocyte-endothelial cell interactions in vivo. *J Am Soc Nephrol*, 19(5), 973–84.

Ntatsaki, E., Watts, R. A., and Scott, D. G. (2010). Epidemiology of ANCA-associated vasculitis. *Rheumat Dis Clin North Am*, 36(3), 447–61.

O'Donnell, J. L., Stevanovic, V. R., Frampton, C., *et al.* (2007). Wegener's granulomatosis in New Zealand: evidence for a latitude-dependent incidence gradient. *Intern Med J*, 37(4), 242–6.

Olson, S. W., Arbogast, C. B., Baker, T. P., *et al.* (2011). Asymptomatic autoantibodies associate with future anti-glomerular basement membrane disease. *J Am Soc Nephrol*, 22(10), 1946–52.

Pagnoux, C., Seror, R., Henegar, C., *et al.* (2010). Clinical features and outcomes in 348 patients with polyarteritis nodosa: a systematic retrospective study of patients diagnosed between 1963 and 2005 and entered into the French Vasculitis Study Group Database. *Arthritis Rheum*, 62(2), 616–26.

Pendergraft, W. F., 3rd, Preston, G. A., Shah, R. R, *et al.* (2004). Autoimmunity is triggered by cPR-3(105–201), a protein complementary to human autoantigen proteinase-3. *Nat Med*, 10(1), 72–9.

Pepper, R. J., Chanouzas, D., Tarzi, R., *et al.* (2013). Intravenous cyclophosphamide and plasmapheresis in dialysis-dependent ANCA-associated vasculitis. *Clin J Am Soc Nephrol*, 8(2), 219–24.

Pepper, R. J., Hamour, S., Chavele, K. M., *et al.* (2013). Leukocyte and serum calprotectin (S100A8/S100A9) expression reflect disease activity in ANCA associated vasculitis and glomerulonephritis. *Kidney Int*, 83(6), 1150–8.

Pepper, R. J. and Salama, A. D. (2012). Classifying and predicting outcomes in ANCA-associated glomerulonephritis. *Nephrol Dial Transplant*, 27(6), 2135–7.

Pfister, H., Ollert, M., Frohlich, L. F., *et al.* (2004). Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. *Blood*, 104(5), 1411–18.

Pinching, A. J., Rees, A. J., Pussell, B. A., *et al.* (1980). Relapses in Wegener's granulomatosis: the role of infection. *Br Med J*, 281(6244), 836–8.

Popa, E. R., Franssen, C. F., Limburg, P. C., *et al.* (2002). In vitro cytokine production and proliferation of T cells from patients with anti-proteinase 3- and antimyeloperoxidase-associated vasculitis, in response to proteinase 3 and myeloperoxidase. *Arthritis Rheum*, 46(7), 1894–904.

Radford, D. J., Luu, N. T., Hewins, P., *et al.* (2001). Antineutrophil cytoplasmic antibodies stabilize adhesion and promote migration of flowing neutrophils on endothelial cells. *Arthritis Rheum*, 44(12), 2851–61.

Saint Marcoux, B. and De Bandt, M. (2006). Vasculitides induced by TNFalpha antagonists: a study in 39 patients in France. *Joint Bone Spine*, 73(6), 710–13.

Salama, A. D., Cook, H. T., Pusey, C. D., *et al.* (2008). A case of treated ANCA-associated vasculitis with recurrent renal failure. *Clin J Am Soc Nephrol*, 3(2), 637–45.

Sangaletti, S., Tripodo, C., Chiodoni, C., *et al.* (2012). Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. *Blood*, 120(15), 3007–18.

Savage, C. O., Pottinger, B. E., Gaskin, G., *et al.* (1992). Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Am J Pathol*, 141(2), 335–42.

Savige, J., Gillis, D., Benson, E., *et al.* (1999). International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). *Am J Clin Pathol*, 111(4), 507–13.

Savige, J., Nassis, L., Cooper, T., *et al.* (2002). Antineutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis after immunisation with bacterial proteins. *Clin Exp Rheumat*, 20(6), 783–9.

Schebendach, J., Mayer, L. E., Devlin, M. J., *et al.* (2012). Dietary energy density and diet variety as risk factors for relapse in anorexia nervosa: a replication. *Int J Eat Disord*, 45(1), 79–84.

Schena, F. P. (1997). Survey of the Italian Registry of Renal Biopsies. Frequency of the renal diseases for 7 consecutive years. The Italian Group of Renal Immunopathology. *Nephrol Dial Transplant*, 12(3), 418–26.

Schlesier, M., Kaspar, T., Gutfleisch, J., *et al.* (1995). Activated CD4+ and CD8+ T-cell subsets in Wegener's granulomatosis. *Rheumatol Int*, 14(5), 213–19.

Schreiber, A., Busjahn, A., Luft, F. C., *et al.* (2003). Membrane expression of proteinase 3 is genetically determined. *J Am Soc Nephrol*, 14(1), 68–75.

Schreiber, A., Xiao, H., Falk, R. J., *et al.* (2006). Bone marrow-derived cells are sufficient and necessary targets to mediate glomerulonephritis and vasculitis induced by anti-myeloperoxidase antibodies. *J Am Soc Nephrol*, 17(12), 3355–64.

Schreiber, A., Xiao, H., Jennette, J. C., *et al.* (2009). C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *J Am Soc Nephrol*, 20(2), 289–98.

Shankarkumar, U., Ghosh, K., Pradhan, V. D., *et al.* (2005). Immunogenetic association in patients with antineutrophil cytoplasmic antibodies (ANCA) from Mumbai, Maharashtra, India. *J Autoimmune*, 24(3), 227–33.

Shaw, G., Ronda, N., Bevan, J. S., *et al.* (1992). Antineutrophil cytoplasmic antibodies (ANCA) of IgA class correlate with disease activity in adult Henoch-Schonlein purpura. *Nephrol Dial Transplant*, 7(12), 1238–41.

Silva, F., Specks, U., Sethi, S., *et al.* (2009). Successful pregnancy and delivery of a healthy newborn despite transplacental transfer of antimyeloperoxidase antibodies from a mother with microscopic polyangiitis. *Am J Kidney Dis*, 54(3), 542–5.

Sinico, R. A., Di Toma, L., Maggiore, U., *et al.* (2006). Renal involvement in Churg-Strauss syndrome. *Am J Kidney Dis*, 47(5), 770–9.

Soto, M. E., Espinola, N., Flores-Suarez, L. F., *et al.* (2008). Takayasu arteritis: clinical features in 110 Mexican Mestizo patients and cardiovascular impact on survival and prognosis. *Clin Exp Rheumat*, 26(3 Suppl 49), S9–15.

Stassen, P. M., Derks, R. P., Kallenberg, C. G., *et al.* (2008). Venous thromboembolism in ANCA-associated vasculitis—incidence and risk factors. *Rheumatology (Oxford)*, 47(4), 530–4.

Stegeman, C. A., Tervaert, J. W., de Jong, P. E., *et al.* (1996). Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. Dutch Co-Trimoxazole Wegener Study Group. *N Engl J Med*, 335(1), 16–20.

Stegeman, C. A., Tervaert, J. W., Sluiter, W. J., *et al.* (1994). Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener granulomatosis. *Ann Intern Med*, 120(1), 12–17.

Stone, J. H., Merkel, P. A., Spiera, R., *et al.* (2010). Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med*, 363(3), 221–32.

Tadema, H., Abdulahad, W. H., Lepse, N., *et al.* (2011a). Bacterial DNA motifs trigger ANCA production in ANCA-associated vasculitis in remission. *Rheumatology (Oxford)*, 50(4), 689–96.

Tadema, H., Kallenberg, C. G., Stegeman, C. A., *et al.* (2011b). Reactivity against complementary proteinase-3 is not increased in patients with PR3-ANCA-associated vasculitis. *PLoS One*, 6(3), e17972.

Tomasson, G., Grayson, P. C., Mahr, A. D., *et al.* (2012). Value of ANCA measurements during remission to predict a relapse of ANCA-associated vasculitis—a meta-analysis. *Rheumatology (Oxford)*, 51(1), 100–9.

Travis, W. D., Hoffman, G. S., Leavitt, R. Y., *et al.* (1991). Surgical pathology of the lung in Wegener's granulomatosis. Review of 87 open lung biopsies from 67 patients. *Am J Surg Pathol*, 15(4), 315–33.

Tull, S. P., Bevins, A., Kuravi, S. J., *et al.* (2012). PR3 and elastase alter PAR1 signaling and trigger vWF release via a calcium-independent mechanism from glomerular endothelial cells. *PLoS One*, 7(8), e43916.

Von Vietinghoff, S., Busjahn, A., Schonemann, C., *et al.* (2006). Major histocompatibility complex HLA region largely explains the genetic variance exercised on neutrophil membrane proteinase 3 expression. *J Am Soc Nephrol*, 17(11), 3185–91.

Voth, M., Haneder, S., Huck, K., *et al.* (2009). Peripheral magnetic resonance angiography with continuous table movement in combination with high spatial and temporal resolution time-resolved MRA With a total single dose (0.1 mmol/kg) of gadobutrol at 3.0 T. *Invest Radiol*, 44(9), 627–33.

Walsh, M., Flossmann, O., Berden, A., *et al.* (2012). Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum*, 64(2), 542–8.

Wang, J. N., Chiou, Y. Y., Chiu, N. T., *et al.* (2007). Renal scarring sequelae in childhood Kawasaki disease. *Pediatr Nephrol*, 22(5), 684–9.

Watts, R., Lane, S., Hanslik, T., *et al.* (2007). Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. *Ann Rheum Dis*, 66(2), 222–7.

Willcocks, L. C., Lyons, P. A., Rees, A. J., *et al.* (2010). The contribution of genetic variation and infection to the pathogenesis of ANCA-associated systemic vasculitis. *Arthritis Res Ther*, 12(1), 202.

Williams, J. M., Ben-Smith, A., Hewins, P., *et al.* (2003). Activation of the G(i) heterotrimeric G protein by ANCA IgG F(ab')2 fragments is necessary but not sufficient to stimulate the recruitment of those downstream mediators used by intact ANCA IgG. *J Am Soc Nephrol*, 14(3), 661–9.

Xiao, H., Heeringa, P., Hu, P., *et al.* (2002). Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest*, 110(7), 955–63.

Xiao, H., Schreiber, A., Heeringa, .P, *et al.* (2007). Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol*, 170(1), 52–64.

Xing, G. Q., Chen, M., Liu, G., *et al.* (2009). Complement activation is involved in renal damage in human antineutrophil cytoplasmic autoantibody associated pauci-immune vasculitis. *J Clin Immunol*, 29(3), 282–91.

CHAPTER 160

The patient with vasculitis: treatment and outcome

Lorraine Harper and David Jayne

Introduction

Prior to the modern therapeutic era, granulomatosis with polyangiitis (GPA) (formerly known as Wegener granulomatosis) had a mortality of 80% at 1 year, largely due to renal failure (Walton, 1958). Glucocorticoids were first used in GPA and polyarteritis in the late 1940s and a randomized trial of adrenocorticotrophic hormone performed by the Medical Research Council (UK) reported better survival of polyarteritis at 1 year (Collagen Diseases and Hypersensitivity Panel, 1957). A follow-up report at 3 years found no difference in survival due to an excess of deaths related to drug-induced adverse events, highlighting the balance of benefits and harms that have continued in vasculitis therapy. In a retrospective survey of polyarteritis, mortality was lower with combination azathioprine/glucocorticoid therapy than glucocorticoids alone or no therapy (Leib et al., 1979). Cyclophosphamide was first used in GPA by Novack and Pearson in 1967 and subsequently by Fauci and colleagues at the US National Institutes of Health (NIH) (Novack and Pearson, 1971). It became apparent that the combination of cyclophosphamide and glucocorticoids resulted in stable remissions in GPA and polyarteritis and permitted reduction or withdrawal of glucocorticoids. The effectiveness of cyclophosphamide was linked to its ability to induce lymphopenia and, in particular, reduction in B-cell counts (Fauci et al., 1971). A 1992 retrospective study of the NIH experience in GPA, in 135 patients, confirmed the effectiveness of this approach but highlighted the greatly increased frequency of urothelial and lymphoproliferative malignancy attributed to the high cumulative exposure to cyclophosphamide (Hoffman et al., 1992). With the association of antineutrophil cytoplasmic antibody (ANCA) with GPA in 1985 and microscopic polyangiitis (MPA) in 1986, and evidence supporting the pathogenesis of ANCA in small vessel vasculitis, a rationale evolved to combine these disorders in therapeutic studies (van der Woude et al., 1985; Savage et al., 1987). Along with eosinophilic granulomatosis with polyangiitis (EGPA) (formerly known as Churg–Strauss syndrome) they were termed ANCA-associated vasculitis (AAV); however, EGPA patients are usually excluded from AAV trials in view of perceived differences in treatment response and pathogenesis.

The last 20 years have seen attempts to reduce the toxicity of therapy by lowering exposure to cyclophosphamide and glucocorticoids, by the use of alternate immunosuppressives and other therapies, and by adjusting the intensity of therapy to the severity of the clinical presentation (Rasmussen et al., 1995). Although therapeutic monoclonal antibodies were first used in vasculitis in 1990, their use has only recently become widespread with the demonstration of the effectiveness of rituximab (Mathieson et al., 1990; Jones et al., 2010; Stone et al., 2010). Over a similar period, improved tools have been developed to assess the outcomes of therapy that have, in turn, emphasized the frequently poor current outcomes with respect to organ damage, chronic morbidity, and poor quality of life (Bacon et al., 1995).

Approaches to therapy

The goals of therapy are to achieve a remission in disease activity, recover renal function, prevent relapse, and minimize drug toxicity. Definitions of disease activity terminology have been agreed by consensus (Table 160.1) (Hellmich et al., 2007). Current regimens employ high doses of toxic agents to suppress manifestations of disease activity and achieve a 'remission' in order to avoid further vital organ damage, rescue renal function, and reduce constitutional disturbance. This accepts a higher level of drug toxicity. This induction phase lasts 3–6 months and is followed by a longer remission maintenance phase when less toxic therapy is used. Prolonged follow-up is then required to manage the consequences of vasculitic damage, drug toxicity, and increased cardiovascular and malignancy risks. Future goals are safer regimens, more rapid remission induction to minimize tissue damage, better relapse prevention, and restoration of quality of life.

Following the association of ANCA with GPA and MPA, clinical trials have combined the two subgroups (Rasmussen et al., 1995). This has been considered appropriate for renal disease, due to similarities in pathogenesis. However, differences in histopathology, treatment response and outcomes, and differences in genetic associations argue for a revision of this approach (Hauer et al., 2002; Lyons et al., 2012). Attempts have been made to subgroup patients at diagnosis according to disease extent and severity in order to design treatments of ascending strength. The European Vasculitis Study Group (EUVAS) have defined four subgroups and a refractory subgrouping while US investigators have separated presentations into limited or non-severe, and generalized or severe (Table 160.2)

Table 160.1 Consensus disease state definitions

Activity state	Definition
Remission	Absence of disease activity attributable to active vasculitis qualified by the need for ongoing stable relapse prevention therapy. 'Active disease' is not restricted to vasculitis only, but includes other inflammatory features, such as granulomatous inflammation in GPA or tissue eosinophilia in CSS
Response	50% reduction of disease activity score and absence of new manifestations
Relapse	Reoccurrence or new onset of disease attributable to active vasculitis
Major relapse	Reoccurrence or new onset of potentially organ- or life-threatening disease
Minor relapse	Reoccurrence or new onset of disease which is neither potentially organ-threatening nor life-threatening
Refractory disease	1. Unchanged or increased disease activity in acute AAV after at least 4 weeks treatment with standard induction therapy, *or* 2. Lack of response, defined as ≤ 50% reduction in the disease activity score, after at least 6 weeks of treatment, *or* 3. Chronic, persistent disease defined as the presence of at least one major or three minor items on the disease activity score list, after ≥ 12 weeks of treatment
Low-activity disease state	Persistence of minor symptoms (e.g. arthralgia, myalgia) that respond to a modest glucocorticoid increase and do not necessarily warrant an escalation of other therapies

CSS = Churg–Strauss syndrome; GPA = granulomatosis with polyangiitis.

From Hellmich et al. (2007).

(Carrington and Liebow, 1966; Rasmussen et al., 1995; Wegener's Granulomatosis Etanercept Trial (WGET) Research Group, 2005). The French Vasculitis Study Group have identified factors predicting increased mortality to develop the five factor score and protocols for patients with good or poor prognosis (Guillevin et al., 2011). However, avoiding diagnostic delay and the early institution of therapy are of primary importance in all subgroups.

Renal vasculitis causing a glomerulonephritis is also seen in immune complex vasculitis (immunoglobulin A (IgA) vasculitis (Henoch–Schönlein) and cryoglobulinaemia), and vasculitis involving larger renal arteries occurs in polyarteritis nodosa and Takaysu arteritis. Less evidence exists to guide therapy in these disorders.

Treatment

Induction therapy

The combination of cyclophosphamide and high-dose glucocorticoids, introduced over 40 years ago, remains the 'standard of care' for new patients with AAV (Mukhtyar et al., 2008). Cyclophosphamide is equally effective as a daily oral or pulsed intravenous (IV) administration for the induction of remission (Table 160.3) (de Groot et al., 2009). However, the IV protocols expose the patient to a lower cumulative cyclophosphamide dose and permit bladder protection. Leucopenia is more common with daily oral cyclophosphamide and is an important risk factor for severe infection (Little et al., 2010). Weekly monitoring of the full blood count is required to detect a falling white count and appropriate dose adjustment of daily oral cyclophosphamide is required to avoid leucopenia. The leucocyte nadir after IV cyclophosphamide occurs at 10–14 days and subsequent cyclophosphamide dosing is reduced if leucopenia occurs. Cyclophosphamide is continued for 3–6 months, by which time remission will have been achieved in 80–90%. IV methyl prednisolone is widely used for severe presentations at total doses of 1000–3000 mg without a robust evidence base (Bolton and Couser, 1979). Daily oral prednisolone commences at 1.0 mg/kg/day, maximum 80 mg/day, for 1–4 weeks, then reduces in steps to 10–15 mg/day by 12 weeks (Jayne et al., 2003; WGET Research Group 2005).

Severe presentations

Plasma exchange improves the chances of renal recovery in those presenting in renal failure, creatinine above 500 μmol/L but does not influence the high mortality risk of this subgroup (Jayne et al., 2007). A meta-analysis failed to confirm a significant benefit of plasma exchange on the composite outcome of death and end-stage renal disease (ESRD) (Walsh et al., 2010). There is also evidence

Table 160.2 Disease subgrouping, US and EUVAS systems, according to disease severity and extent

Subgrouping by severity or extent		Organ involvement	Constitutional symptoms	ANCA status	Serum creatinine (μmol/L)
US	**EUVAS**				
Limited or non-severe	Localized	One site, typically the upper respiratory tract in GPA	No	Positive or negative	< 120
	Early systemic	Any, except renal or imminent vital organ failure	Yes	Positive	< 120
Generalized or severe	Generalized (or renal)	Imminent vital organ failure or renal vasculitis	Yes	Positive	< 500
	Severe	Vital organ failure, typically renal	Yes	Positive	> 500 (renal presentations)
	Refractory	Progressive disease despite conventional therapy	Yes	Positive or negative	Any

From Carrington and Liebow et al. (1966) and Rasmussen et al. (1995).

Table 160.3 Cyclophosphamide dosing for IV pulsed and daily oral regimens as defined in the EUVAS CYCLOPS protocol

Pulsed cyclophosphamide dosing (mg/kg/pulse) with reductions for impaired renal function and age		
Age (years)	**eGFR > 30 mL/min**	**eGFR ≤ 30 mL/min**
< 60	15	12.5
60–70	12.5	10
> 70	10	7.5
Timing of cyclophosphamide pulses		
Weeks: 0, 2, and 4, then every 3 weeks (weeks 7, 10, 13, 16, 19, 21, and 24)		
Daily oral cyclophosphamide dosing		
Age (years)	Dose (mg/kg/day)	
< 60	2.0	
60–70	1.5	
> 75	1.0	

eGFR = estimated glomerular filtration rate.

From de Groot et al. (2009).

that plasma exchange improves renal outcomes in renal vasculitis with deteriorating renal function below a creatinine of 500 μmol/L, but this requires confirmation (Gregersen et al., 2012). The increasing evidence for the pathogenicity of ANCA in renal vasculitis provides a rationale for its use, but removal of coagulation factors, cytokines, or other substances may also be important. Pulmonary haemorrhage results from an alveolar capillaritis and is treated in a similar manner to renal vasculitis (Kostianovsky et al., 2012). Plasma exchange has been recommended for severe haemorrhage with hypoxia on the basis of uncontrolled studies. Exacerbation of bleeding by removal of coagulation factors is a theoretical concern and confirmation of efficacy is needed. Plasma filtration or centrifugation appears equally effective and, on average, seven daily or alternate day exchanges of 1–1.5 plasma volumes are used. Double filtration apheresis, selective IgG, and myeloperoxidase (MPO)-ANCA selective immunoabsorption have also been employed but until there is better understanding of the mechanisms of plasma exchange, non-selective procedures are preferred. The procedure usually requires central vascular access, and may be complicated by haemorrhage and thrombocytopaenia. Volume replacement with albumin is recommended but plasma or coagulation factor-rich plasma fractions are used in the setting of increased bleeding risk, such as after a renal biopsy or in the presence of alveolar haemorrhage.

Non-severe presentations

Cyclophosphamide substitution by methotrexate, dosed at 20–25 mg/week, for non-severe AAV presentations was found to be effective in the NORAM trial with similar remission rates at 6 months (de Groot et al., 2005). One-third of patients in this trial had haematuria with normal renal function indicating early renal vasculitis. Enthusiasm for this approach has waned with a higher relapse rate following methotrexate induction and higher subsequent glucocorticoid and cyclophosphamide requirement (Faurschou et al., 2012). Mycophenolate mofetil has been shown to have potential as an alternative to cyclophosphamide in MPO-ANCA-positive MPA in preliminary studies (Hu et al., 2008; Silva et al., 2010).

Rituximab

B-cell depletion with rituximab is as effective as cyclophosphamide for the induction of remission in AAV (Jones et al., 2010; Stone et al., 2010). The RITUXVAS trial employed two cyclophosphamide pulses with rituximab 375 mg/m^2/week × 4, but the results in the RAVE trial, with the same rituximab dose but no cyclophosphamide, were similar. No early benefits of cyclophosphamide avoidance were observed in either trial. Rituximab can be recommended for remission induction when cyclophosphamide is contraindicated, for example, by infection, cytopenia, intolerance, malignancy, or fertility protection (Guerry et al., 2012). A simpler regimen of rituximab 1 g repeated after 14 days appears to be similar in efficacy to the four-dose regimen (Jones et al., 2009). Relapse is frequent after rituximab with 26% in the RITUXVAS trial and 40% in the RAVE trial relapsing in the second year, relapse being more common in those with a history of relapse. The time to achieve remission of renal vasculitis, and the renal outcomes were the same for rituximab or cyclophosphamide in the RAVE trial. Concomitant initial cyclophosphamide was used with rituximab in the RITUXVAS trial but not in the RAVE trial. The latter excluded patients with creatinine > 360 μmol/L, but extrapolation suggests routine cyclophosphamide is not required with rituximab for the more severe presentations. Because rituximab is removed by plasma exchange when both therapies are employed, at least 48 hours should elapse between rituximab infusion and the next plasma exchange and at least 50% of the rituximab total dose should be administered after the plasma exchange course has been completed.

Treatment in other forms of renal vasculitis

Immune complex vasculitis

IgA vasculitis in children remits spontaneously and therapy is only indicated in the more severe cases. Adolescents and adults are more likely to have a persistent or relapsing course of disease, however many will spontaneously remit and non-severe cases do not require treatment. High-dose glucocorticoids are widely used and plasma exchange has a rationale based on the removal of circulating IgA or IgA-containing immune complexes. There is support for plasma exchange and IV immunoglobulin from cohort studies. It is unclear whether immunosuppressives, including cyclophosphamide or azathioprine, contribute to remission induction in this disease with one small randomized trial reporting no benefit with cyclophosphamide in severe renal or gastrointestinal IgA vasculitis (Pillebout et al., 2010).

Polyarteritis nodosa has been treated with IV cyclophosphamide and high-dose glucocorticoids as for AAV. Remission and relapse rates are also similar (Guillevin et al., 2003). Results of uncontrolled studies suggest that hepatitis B virus (HBV)-associated polyarteritis nodosa should be treated with antiviral agents, short-term corticosteroids, and plasma exchange. Relapse in HBV-associated polyarteritis nodosa is rare and never occurs once viral replication is stopped and seroconversion occurs (Guillevin et al., 2005). The management of Takayasu renal arteritis includes endovascular procedures including angioplasty or surgical bypass and assessment and control of inflammatory disease by glucocorticoids

with or without immunosuppressives or anti-cytokine antibodies (Mukhtyar et al., 2008).

Assessing response

Control of renal vasculitis is judged by improvement or stability of renal function, absence of extrarenal vasculitis, and normalization of C-reactive protein (CRP). Microscopic haematuria persists for many months after the onset of therapy and proteinuria typically increases during the recovery period, occasionally to levels sufficient to cause the nephrotic syndrome especially in those uncommon patients with evidence of immune complex deposition. The level of proteinuria during the recovery phase indicates the degree of glomerular injury and is associated with worse renal outcomes (Bakoush et al., 2006). The significance of persistent low-level haematuria is unclear. Repeat renal biopsy in the presence of ≥ 2+ by urine dipstick or > 30 red cells/high powered field is usually associated with active lesions on biopsy. The value of ANCA levels to guide the duration or intensity of induction therapy has not been assessed and has not been of prognostic significance. For patients who are dialysis dependent at presentation, if recovery occurs it is usually seen within 2–3 weeks, although later renal recovery can occur. If no recovery has been seen after a course of plasma exchange has been completed, a persisting high ANCA level, high CRP attributed to vasculitis, or poor control of extrarenal vasculitis suggest that renal vasculitis is likely to be still active and further glucocorticoid and plasma exchange would be beneficial. However, if there is no recovery by 4–6 weeks further intensive therapy is likely to be unproductive. The severity of the renal histology at diagnosis should not deter the commencement of induction therapy; however, repeat renal biopsy can be useful in refractory cases when the presence of active vasculitis and degree of sclerosis would influence subsequent treatment decisions. For those presenting with renal-limited vasculitis, induction therapy can be withdrawn if there is no recovery by 12 weeks.

Induction treatment in children and the elderly

The approach to therapy and responsiveness to medication is the same in the young and the old to those in other age groups but drug selection and dosing may differ. In view of the fertility and malignancy risks of cyclophosphamide, alternative use of mycophenolate mofetil or rituximab has been suggested. Higher glucocorticoid doses, up to 2 mg/kg/day, are used in children due to increased rates of elimination. The elderly are more likely to present with renal impairment and have a high risk of infective complications. It is important to reduce cyclophosphamide accordingly. Lower cyclophosphamide dosing, fixed at 500 mg IV pulses, and reduced glucocorticoid exposure with more rapid tapering have been proposed. Glucocorticoid withdrawal in the remission period is desirable to reduce the risk of osteoporosis.

Remission maintenance therapy

Disease relapse occurs in 75% of GPA and 30% of MPA and EGPA cases by 5 years (Booth et al., 2003; Walsh et al., 2012). Predictors of relapse include anti-proteinase 3 (PR3) antibodies, lung and upper respiratory involvement (Hogan et al., 1996), age (Koldingsnes et al., 2002), nasal carriage of *Staphylococcus aureus* (Stegeman et al., 1994), and absence of severe renal involvement (Koldingsnes and Nossent, 2002; Pierrot-Deseilligny Despujol et al., 2010). Granulomatous relapses are more frequent and earlier than those with predominant vascular disease (Pierrot-Deseilligny Despujol et al., 2010). Patients with ESRD have low rates of relapse (Weidanz et al., 2007; Little et al., 2010). Lower cyclophosphamide exposure during induction therapy increases later relapse risk (Harper et al., 2012). A $CD8^+$ T-cell transcription signature has been identified that can predict patients at risk of relapse. The subset of genes defining the poor prognostic group were enriched for genes involved in the interleukin (IL)-7 receptor pathway, T-cell receptor signalling, and genes expressed by memory T cells (McKinney et al., 2010).

Changes in ANCA do not correlate closely with disease activity and rises should not trigger increases in therapy on their own. A persistent ANCA after induction therapy, a rise in ANCA during remission therapy, and ANCA positivity at the time of treatment withdrawal indicate a higher relapse risk (McKinney et al., 2006; Tomasson et al., 2012) (Table 160.4).

The goals of maintenance therapy are to prevent disease relapse while minimizing drug toxicity. Cyclophosphamide is withdrawn after 3–6 months and substituted by azathioprine 2 mg/kg/day, or methotrexate, up to 25 mg/week. They were equally effective for remission maintenance with similar safety risks in the WEGENT trial (Pagnoux et al., 2008). Methotrexate is excreted by the kidneys and should be avoided in the presence of renal impairment, glomerular filtration rate (GFR) < 50 mL/min. Although rare, concerns over methotrexate pneumonitis complicate its use in the presence of pulmonary vasculitis. Leflunomide, 20 mg/day, was superior to methotrexate for the prevention of relapse in a small GPA study; however, this was at the expense of an increased frequency of adverse events. Mycophenolate mofetil was less effective than azathioprine for relapse prevention after cyclophosphamide induction therapy (Metzler et al., 2007; Hiemstra et al., 2010). Mycophenolic acid plasma level monitoring may increase the safety and efficacy of mycophenolate mofetil but this requires confirmation in vasculitis.

Prednisolone is either continued in conjunction with an immunosuppressive at doses of 5–10 mg/day or is withdrawn at the end of the induction phase. There is an increased relapse risk following steroid withdrawal which has to be balanced against the toxicity of long-term administration (Walsh et al., 2010). Long-term steroids are often required in patients with EGPA due to persistent asthma.

There is no clear guidance as to how long remission maintenance treatment should be continued. The IMPROVE trial withdrew therapy 42 months after diagnosis, but withdrawal after 24 months has also been suggested. The various factors that influence relapse risk should be considered (Table 160.4) along with the likely consequences of relapse for the individual patient. Most relapses are minor and do not increase the risk of death or organ failure,

Table 160.4 Factors increasing relapse risk in ANCA-associated vasculitis

Clinical presentation	Serology	Treatment related
Diagnosis of GPA (Wegener's)	PR3-ANCA positive at diagnosis	Steroid withdrawal
Ear, nose and throat involvement	Persistent ANCA positivity after induction therapy	Immunosuppressive withdrawal
Serum creatinine < 200 μmol/L	Rise in ANCA during remission	Lower cyclophosphamide exposure

but delayed relapse diagnosis is a real concern and renal relapse increases the risk of ESRD. There should be consideration for prolonged low dose immunosuppression in cases at high risk of relapse. Regular monitoring and patient education will permit relapse to be detected early, especially if therapy has been withdrawn.

Relapse is frequent after rituximab, with 26% in the RITUXVAS trial and 40% in the RAVE trial relapsing in the second year, and is more frequent in patients with a history of relapse. There are no current recommendations for remission management after rituximab with patients either being observed and re-treated with rituximab at the time of relapse, being administered rituximab at fixed intervals or on the basis of ANCA levels or B-cell counts, or a conventional immunosuppressive, such as azathioprine, used (Cartin-Ceba et al., 2012; Smith et al., 2012). Immunosuppressives did not reduce the subsequent relapse rate in one multicentre survey, and relapses can occur without B-cell repopulation or rises in ANCA. Fixed-interval repeat-dose rituximab, 1000 mg every 6 months for 2 years, has reduced relapse rates when rituximab is used in relapsing patients (Smith et al., 2012).

Management of relapse

The symptoms and signs of relapse in an individual patient reflect those present prior to the original diagnosis. The diagnosis of relapse needs to be differentiated from infection or other potential causes, including malignancy. Infection may precede and precipitate relapse and this is a particular issue with bacterial infections in respiratory tract relapse in GPA. Relapse is categorized as minor (non-severe), or major (severe) when vasculitic activity threatens vital organ function. Renal relapse is initially manifested by a return or increase in haematuria with proteinuria with subsequent deterioration in renal function and is usually, but not always, associated with ANCA positivity and rises in erythrocyte sedimentation rate and CRP. Repeat renal biopsy is indicated if there is uncertainty as to whether or not renal relapse is occurring.

Minor relapse is treated by optimization of the background immunosuppressive, such as azathioprine, and increase in prednisolone to 0.5–1.0 mg/kg/day with a reducing regimen back to 5–10 mg/day. When the immunosuppressive dose is limited by adverse events, or if minor relapses recur, an alternative immunosuppressive or switch to rituximab should be considered. Major relapse is treated by the introduction of cyclophosphamide or rituximab and a similar increase in prednisolone (Mukhtyar et al., 2008). The RAVE trial found a higher rate of response to rituximab than to cyclophosphamide in relapsing patients and rituximab is now recommended for this subgroup (Stone et al., 2010; Guerry et al., 2012).

Refractory disease

Progression of vasculitis despite induction therapy, failure to attain disease remission, and disease relapse while receiving maintenance therapy are defined as refractory disease (Table 160.3) (Hellmich et al., 2007). Before therapy is enhanced, causes for refractory disease, including infection, malignancy, and drugs, should be considered as well as non-concordance with the prescribed regimen. Drug intolerance, especially to glucocorticoids or cyclophosphamide, and reductions in dosing due to intercurrent infection may also lead to primary treatment failure. This situation is associated with a high mortality due to the presence of organ failure and the risks of prolonged therapy (Seror et al., 2010).

Progressive or non-responsive disease occurs in 5–10% and is treated with an increase in glucocorticoid, typically IV pulsed methylprednisolone 1000–3000 mg. Relative cyclophosphamide underdosing is indicated by a failure to induce lymphopenia and is more common with IV pulsed administration. A switch to daily oral cyclophosphamide or an increase in IV dosing has been suggested. Changing from cyclophosphamide to rituximab is now more attractive because rituximab is less likely to increase the infective risk. Failure to induce B-cell depletion indicates that rituximab will be ineffective. Where the response to rituximab appears slow, pulse cyclophosphamide can be added until a response is seen, but cyclophosphamide is not routinely required with rituximab.

Plasma exchange can be considered for resistant renal vasculitis or alveolar haemorrhage, and IV immunoglobulin reduces disease activity in refractory AAV but the effect lasts less < 3 months (Jayne et al., 2000). This option can be considered if conventional therapy is contraindicated, for example, by infection or in pregnancy. A potential mechanism of immunoglobulin, as proposed in Kawasaki disease, is the neutralization of microbial toxins. Blockade of tumour necrosis factor alpha (TNF-α) with infliximab or etanercept has led to remission when used as an additional agent but prolonged use appears ineffective and it may increase the risks of infection (Booth et al., 2004a; WGET Research Group, 2005). Concerns over malignancy risk with anti-TNF therapy in vasculitis have not been supported by longer-term studies (Silva et al., 2011).

Patients failing to achieve remission by 6 months, or possibly before, should have their non-glucocorticoid treatment reassessed as for progressive disease. Deoxyspergualin (gusperimus) is an immunosuppressive with a range of activity on the innate and cognate immune systems that has demonstrated high response levels in refractory GPA (Flossmann et al., 2009). Leucopenia is common but rapidly reversible and not accompanied by increased infection frequency. There does not appear to be a sustained effect after deoxyspergualin withdrawal when relapses are common. Lymphocyte, eosinophil, and macrophage depletion with the anti-CD52 therapeutic antibody alemtuzumab has led to sustained treatment-free remissions (Walsh et al., 2008). The profound, transient lymphopenia induced by alemtuzumab is poorly tolerated in those > 60 years or with impaired renal function.

Those relapsing are treated as described above with multiple minor relapses or at least one major relapse requiring a change of immunosuppressive. Changing the non-cyclophosphamide immunosuppressive, for example, from azathioprine to methotrexate, or vice versa, or switching to mycophenolate mofetil or leflunomide, can be considered (Koukoulaki and Jayne, 2006; Stassen et al., 2007). After a major relapse or after a failure of at least one alternative immunosuppressive for minor relapses, rituximab is indicated.

Management of damage

Chronic kidney disease resulting from renal vasculitis is managed in a similar way to other causes of renal disease. Proteinuria rises during the recovery phase of renal vasculitis reflecting glomerular damage. Angiotensin-converting enzyme inhibition has been recommended to improve long-term renal outcomes without direct supporting evidence. Patients with reduced renal function are at high risk of ESRD if they have a further episode of renal vasculitis and need to be monitored with this in mind. Those developing ESRD can be supported by dialysis and their immunosuppressive treatment withdrawn in the absence of extrarenal disease.

Peritoneal dialysis carries an increased risk of peritonitis and need for ongoing immunosuppression is a relative contraindication. Patients who develop ESRD should be considered for transplantation, which is a safe and effective option (Geetha et al., 2011). It has been conventional to wait at least 6 months after an episode of active vasculitis before transplanting. ANCA positivity at the time of transplantation does not appear to affect graft or patient survival, although an increase in graft vasculopathy was noted in one series (Little et al., 2009).

Ear, nose, and throat (ENT) disease in GPA has a high risk of causing irreversible damage and chronic symptomatology. Collapse of the bridge of the nose can be corrected by bone or cartilage grafts and restorative procedures should be performed when the disease is thoroughly controlled and glucocorticoid doses at remission maintenance levels. Damage to the nasal mucosa results in nasal crusting which provides a focus for infection as well as causing nasal obstruction. Regular nasal douching to remove the crusts and use of a topical aseptic cream, such as mupirocin, reduces associated symptoms and risk of infection (Laudien et al., 2009). Hearing loss is usually caused by Eustachian tube obstruction and recurrent otitis media but may also result from damage to the eighth cranial nerve. Although some recovery may occur, chronic hearing loss is frequent. Subglottic, tracheal, or bronchial stenoses can be fibrotic in origin reflecting previous episodes of vasculitis damage to the airways. They can progress to cause respiratory obstruction or, if at bronchial level, cause segmental or lobar obstruction, infection, and collapse. Physical dilatation by bougie or balloon can restore the lumen and antibiotics are usually required to control secondary infection. Multiple procedures may be required as well as subsequent intermittent surveillance by nasendoscopy or bronchoscopy.

The long-term outcome of alveolar haemorrhage is usually good with stable lung function but a minority of patients present with overlapping features of ANCA vasculitis and pulmonary fibrosis (Hervier et al., 2009; Arulkumaran et al., 2011). The lung fibrosis can precede or follow vasculitis and be progressive leading to respiratory failure despite control of vasculitis. This pattern is more commonly associated with the MPO-ANCA subtype.

Adverse events

Improvement in survival has resulted from modern treatment strategies; however, adverse events associated with these treatments remain an important cause of death and morbidity. In the EUVAS studies mortality at 1 year was 11%. The cause of death was related to an adverse event of treatment in 59%, with infection being the most common factor (Fig. 160.1) (Little et al., 2010). Active vasculitis only accounted for 14% of deaths. This study highlights the burden of using non-selective immunosuppressant agents. Infection is also a common cause of morbidity with 25% of patients developing infection in the first year, with respiratory tract and generalized septicaemia being the most common infections. Glucocorticoid and cytotoxic therapy both contribute to the increased risks of infection. An older study of 158 patients over a median of 8 years, which used prolonged oral cyclophosphamide, found that 50% of serious bacterial, fungal, and opportunistic infections occurred during periods of daily glucocorticoid therapy, with only 21% occurring on alternate-day glucocorticoids, 16% on single-agent cytotoxic therapy, and 12% whilst therapy free (Hoffman et al., 1992).

Infections

Those at risk of infection should be identified and interventions undertaken to reduce risk. Factors predictive of infection include age, severity of renal dysfunction, leucopenia, and intensity and duration of immunosuppression. *Pneumocystis jirovecii* infection occurs in AAV with an incidence of 0.85–12% usually within the first few months after diagnosis, when immunosuppressive therapy is most intense. Presentation is more acute than that commonly seen in HIV patients, with 41% having symptom duration of 3 days or less and high fevers with rapid onset of respiratory failure being common. Mortality rates of 47–62.5% are reported. *Pneumocystis* infection is associated with lymphopenia, oral cyclophosphamide, and steroid use. Prophylaxis against *Pneumocystis jirovecii* with low-dose sulphamethoxazole/trimethoprim is recommended in patients receiving cyclophosphamide (Mukhtyar et al., 2008). Herpes zoster varicella (HZV), shingles, occurs in AAV with a frequency of 4.5 episodes per 100 patient years. While viral

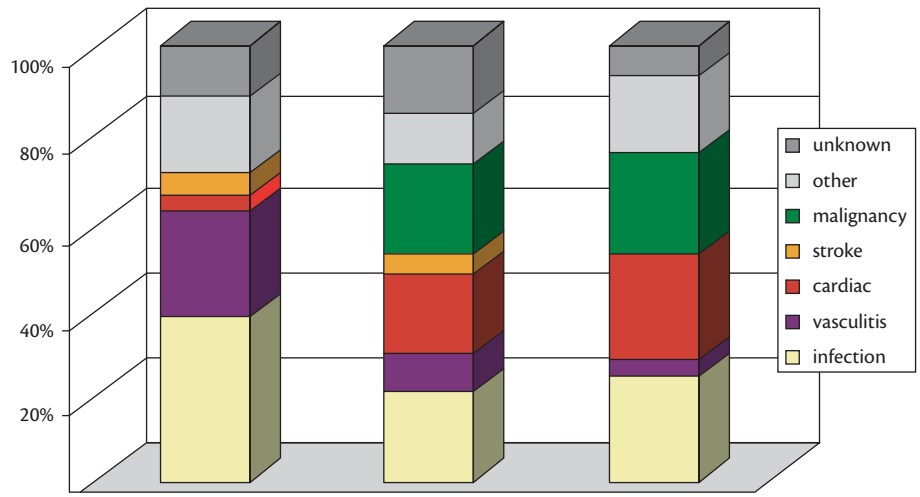

Fig. 160.1 Causes of death in 535 patients enrolled at diagnosis into prospective EUVAS trials in the first year, the second to fifth year, and after 5 years.
From Flossmann et al. (2011).

reactivation is linked to the intensity of immunosuppression, infections also occur following the switch to maintenance therapy.

Leucopenia is common, especially in those receiving oral cyclophosphamide, and is directly associated with infection and death (Booth et al., 2003). It should be avoided with close monitoring. Leucopenic patients can be treated with granulocyte-colony stimulating factor although in a mouse model this has associated with exacerbation of disease (Freeley et al., 2013). Influenza immunization was safe and effective in 230 GPA patients and is recommended for AAV patients (Stassen et al., 2008). Pneumococcal vaccination is recommended but response rates are often poor when using the polysaccharide vaccine, Pneumovax®. Live vaccines should be avoided in all patients taking immunosuppressives or prednisolone doses > 5 mg/day. In those AAV patients on standard maintenance therapy, maintenance of protective levels of antibody to other vaccines such as diphtheria, tetanus, and polio may be shorter than in immunocompetent patients and may require more frequent booster vaccinations (van Assen et al., 2011).

Malignancy

The incidence of cancer in treated AAV patients is 1.6–2.4 times higher than that of the general population. Immunosuppressive drugs contribute to this risk, especially a 4.8–33-fold risk of urothelial cancer and 4.2–11-fold risk of leukaemia or lymphoma, attributed to cyclophosphamide, but proportionally higher rates of lymphoma and non-melanoma skin also occur (Hoffman et al., 1992). Data collected from the EUVAS studies on patients recruited between 1995 and 2003 found an increased risk of all cancers of 1.7, largely driven by non-melanoma skin cancer (Heijl et al., 2011). The previously observed increased risk of bladder cancer and leukaemia was not seen, reflecting the reduced cyclophosphamide exposure in the EUVAS protocols. The low relative risk and bladder cancer frequency seen in this study may have been a feature of relatively short follow-up. A French cohort study found a fivefold increase in risk for bladder cancer in AAV patients who had received cyclophosphamide, with cumulative cyclophosphamide dose and oral cyclophosphamide treatment being important risk factors. A single-centre Danish cohort study of 293 GPA patients recruited from 1973 to 1999 found an overall increased risk of 2.1, rising to 3.6 for bladder cancer and leukaemia, with no increased risk for these cancers in those receiving a cyclophosphamide exposure of < 36 g (Faurschou et al., 2008). It appears probable that the bladder cancer risk is associated with cyclophosphamide exposure and length of follow-up, and strategies such as IV pulse administration with hydration and mesna will minimize this risk. However, patients who have received cyclophosphamide require lifelong follow-up with prompt investigation by cystoscopy and urine cytology for the new onset or persistence of haematuria. Azathioprine increases the risk of skin malignancy and may contribute to lymphoma risk. Increased risks are detectable with > 3 years' exposure in inflammatory bowel disease but have not been quantified in vasculitis. Patients receiving azathioprine should receive appropriate advice to minimize sun exposure and should be counselled and reviewed with these risks in mind.

Cardiovascular disease

Myocardial infarction rates were increased with a hazard ratio of 3.6 in a retrospective review of GPA patients enrolled in the Danish National Hospital Register. In addition, a retrospective study showed that patients with GPA and MPA, when matched for renal function and other traditional risk factors, had double the rate of cardiovascular events. Of 535 GPA and MPA patients enrolled in the EUVAS trials, 74 (14%) had had a cardiovascular event by 5 years, an increased risk of 3.7 (Suppiah et al., 2011). Those who were MPO-ANCA positive had higher risk than those with PR3-ANCA. Theories for the increased event rates in AAV include systemic inflammation and endothelial dysfunction, factors associated with increased cardiovascular risk in other inflammatory diseases. AAV patients in remission have impaired endothelial function and increased stiffness of large arteries, which has been reversed by TNF-α blockade (Booth et al., 2004b). Renal dysfunction is an independent predictor of cardiovascular disease. Patients with AAV may also have an increased prevalence of traditional risk factors for atherosclerotic disease due to glucocorticoid treatment, which causes hypertension, dyslipidaemia, diabetes, and weight gain.

Thromboembolic disease

Although microthrombosis is a component of vasculitic pathology, the frequency of deep venous thrombosis and pulmonary embolism is also increased, being highest during periods of disease activity. A rate of 15% was reported in a prospective trial and in retrospective surveys rates of 1.8%/year for all AAV patients rising to 6.7–9%/year for those with active disease (Merkel et al., 2005). Autoantibodies to plasminogen and tissue plasminogen activator occur in the sera of 25% and 14% of AAV patients. Their presence has been associated with more severe renal outcomes and increased risk for thromboembolic events. Anticoagulants have been used historically in the treatment of renal vasculitis but thromboprophylactic strategies have not been developed for AAV in the current era. Until further data emerges of the clinical utility of anti-plasminogen autoantibody testing it is advisable to address conventional risk factors and maintain a high threshold for suspicion of thromboembolism.

The coincidence of the pulmonary-renal syndrome, with lung haemorrhage, and thromboembolic disease has been reported in seven patients with AAV. Therapeutic anticoagulation did not appear to exacerbate the lung haemorrhage (De Sousa et al., 2012).

Other drug-specific toxicity

Glucocorticoids

Glucocorticoids have a broad adverse event profile, including steroid-associated diabetes, avascular necrosis, and ocular cataract formation. Prophylaxis against osteoporosis and peptic ulceration has become routine, especially in those receiving high-dose glucocorticoids. Reduced bone mineral density is common in patients with AAV; in one study of 99 patients with AAV, 57% had osteopenia and 21% had osteoporosis in at least one site, seven out of 99 patients sustained fractures. Other studies also report high rates of fractures in AAV patients of 2.5–15%. Bisphosphonates are contraindicated in those with a GFR < 30 mL/min.

Cyclophosphamide

The adverse events specific to cyclophosphamide, other than infection and bone marrow suppression, include hair loss, haemorrhagic cystitis, and infertility. Haemorrhagic cystitis occurs with a frequency of 0.5/100 patient-years and is associated with oral cyclophosphamide use and total exposure. Prevention of haemorrhagic

cystitis is important as an episode increases the risk of bladder cancer by five to seven times. The risk of haemorrhagic cystitis can be reduced by increased hydration and by concomitant treatment with mesna, which binds to the cyclophosphamide toxic metabolite, acrolein. Fertility risks are discussed in 'Fertility and pregnancy'.

Azathioprine

Myelosuppression is common with azathioprine and can occur early or later during its administration. Mutant polymorphisms in the thiopurine S-methyltransferase (TPMT) gene are associated with rapid and profound myelosuppression. Patients can be screened for common TPMT polymorphisms or for the biochemical activity of TPMT before commencing azathioprine. The significance of heterozygous states and borderline low activity levels is less clear as many such patients tolerate azathioprine well. A recent randomized controlled trial investigating whether genotyping prior to starting azathioprine reduced the number of adverse drug reactions showed no benefit (Newman et al., 2011). Azathioprine allergy or intolerance occurs in 5–10% and hypersensitivity reactions can be difficult to distinguish from infection or vasculitic relapse, but their onset within 2–3 weeks of commencing azathioprine is an indicator. Reactions are manifested by fevers, chills, rash, and interstitial nephritis can occur. Hepatotoxicity and cirrhosis are less common.

Rituximab

The use of rituximab is increasing in patients with AAV. No change in infection rates was observed when rituximab was substituted for cyclophosphamide in two induction trials (Jones et al., 2010; Stone et al., 2010). Whether this reflects an infection risk with rituximab similar to cyclophosphamide or the role of concomitant high-dose steroid is unclear. *Progressive multifocal leucoencephalopathy*, caused by the JC virus, has occurred in systemic lupus erythematosus (SLE) patients treated with rituximab but it is unclear whether the prevalence of PML is actually increased by rituximab in SLE. There has not been evidence of increased PML risk with rituximab in AAV but patients should be counselled that such a risk might exist. Infusion reactions to rituximab occur in 20% but have been mild without sequelae and have not prevented repeat treatment. Hypogammaglobulinaemia occurs after rituximab in a minority and is related to the use of previous immunosuppressives, cumulative exposure to rituximab, and length of follow-up, it may be more frequent in those previously treated with cyclophosphamide (Venhoff et al., 2012). While mild reductions of IgG do not appear to influence infective risk, severe deficiency to < 3 g/L has occurred and led to recurrent infection and need for immunoglobulin replacement. Rituximab impairs the humoral response to immunizations, and, where possible, these should be administered at least 2 weeks before, or 4 months after, rituximab.

Fertility and pregnancy

Vasculitis activity and its therapy are threats to the fertility of patients with vasculitis. Loss of fertility is an important consequence of the disease, but the risks of this occurring can be considerably reduced with newer forms of treatment. As a chronic disease, vasculitis also causes psychosexual and relationship problems due to effects on self-esteem and mental well-being. Chronic kidney disease is a common consequence of renal vasculitis and depressed kidney function itself affects fertility in both women and men.

The major threat to fertility is cyclophosphamide exposure that can result in primary ovarian failure. This is related to the total amount of cyclophosphamide administered and the age of the patient. Data from lupus nephritis suggests that a total cyclophosphamide exposure of 14–20 g results in infertility in > 50% of women aged > 32 years (Hickman and Gordon, 2011). The risk of infertility in those < 32 years is lower, around 10% in one series. Even if infertility is not induced, less severe ovarian damage leads to early menopause. Drugs that temporarily suppress ovarian function, such as Zoladex®, are used to reduce the risk of cyclophosphamide toxicity. Rituximab has been shown to be as effective as cyclophosphamide and can be used when cyclophosphamide avoidance is desirable. There have been concerns that cyclophosphamide, through damage to DNA in the unfertilized egg, results in an increase in birth defects but this has not proved to be the case. However, it is advisable to wait at least 6 months between stopping cyclophosphamide and attempting to conceive.

Cyclophosphamide directly affects sperm production in men but there is more potential for recovery by the generation of new sperm-forming cells when cyclophosphamide is withdrawn, although pre-pubertal boys are at greater risk of infertility than girls with a threshold of 200 mg/kg deduced from nephritis trials. Sperm production does not usually recover to pre-treatment levels and healthy sperm counts can remain depressed. It is likely that, in combination with non-specific effects of chronic illness, cyclophosphamide reduces male fertility. An alternative immune suppressive used in vasculitis, methotrexate, also reduces sperm formation but has a lower risk of sustained effects after withdrawal. Egg preservation in women can be difficult due to the urgency in starting therapy but semen preservation is quite feasible in men and can be considered before cyclophosphamide is commenced.

Methotrexate and mycophenolate mofetil, damage the fetus and must not be used in pregnant women or those attempting to conceive. Anti-inflammatory drugs and high-dose steroids also reduce fertility. The infective risks of the coil are increased in those receiving immune suppression. Sexually transmitted diseases can be more problematic in immune suppressed patients and *Chlamydia trachomatis* results in infertility in women. Drug effects, especially high-dose steroids, vasculitic activity, and chronic illness reduce testosterone levels that can lead to reduced libido and erectile failure. Testosterone levels in the blood are readily measured and testosterone supplementation can correct the problem.

GPA has been diagnosed during pregnancy but this is rare and there is no evidence of an increased relapse risk during pregnancy or the post-puerperal period. The risks of pre-existing damage to the pregnancy, in particular to the kidneys, lungs, or heart, need to be considered before conception, and appropriate changes to medication made. Transmission of ANCA and a self-limiting vasculitis syndrome has been reported in the neonate, but this is probably a rare occurrence. The largest review of 22 pregnancies in AAV reported good fetal outcomes, the adverse events being pre-eclampsia in two pregnancies and one newborn with hypothyroidism and one with a cleft palate.

Outcomes

Survival

Over the last 30 years treatment has improved the outcome for patients with AAV. Most patients respond to treatment with 85% of patients achieving remission. Factors predicting treatment resistance include older age (Pagnoux et al., 2008). Patient survival

is reported as 45–91% at 5 years and 75–88% at 10 years compared with 80% mortality at 2 years if left untreated (Mukhtyar et al., 2008).

Despite advances in therapy, patients continue to have a substantially higher mortality than a matched background population as shown by a recent study of long-term outcomes of patients recruited to the EUVAS studies (Flossmann et al., 2011). The mortality rate ratio was 2.6 compared to the normal population, with advanced renal failure, increasing age, and a high Birmingham Vasculitis Activity Score being the main predictors of an adverse outcome. Several other studies have identified increasing age and worsening renal function as poor prognostic markers (Hogan et al., 1996; Reinhold-Keller et al., 2000). Interestingly, in those patients recruited to the EUVAS studies there was no difference in survival between patients with GPA or MPA (Flossmann et al., 2011) in contrast to other studies (Hogan et al., 1996; Weidner et al., 2004). Mortality is highest in the first year with 1-, 2-, and 5-year survival being 88%, 85%, and 78% respectively. Disease and therapy-related deaths, particularly infection, account for the majority of deaths in the first year. Infection remains an important cause of death even beyond 1 year, but malignancy and cardiovascular disease are also common (Flossmann et al., 2011).

Renal outcome

End-stage kidney disease (ESKD) is not uncommon in patients with AAV; approximately 20% of those presenting who have evidence of renal involvement will develop ESKD by 5 years (Nachman et al., 1996; Booth et al., 2003). In a multivariate analysis renal survival was best predicted by presenting serum creatinine and percentage of normal glomeruli in the diagnostic biopsy (de Lind van Wijngaarden et al., 2006; Day et al., 2010). However, even in those presenting with severe histological findings and very low numbers of normal glomeruli, treatment should be given as the chance of renal recovery is greater than for therapy-related death (de Lind van Wijngaarden et al., 2007).

A simple histological classification of ANCA-associated renal vasculitis divides biopsies into focal (> 50% normal glomeruli), crescentic (> 50% glomeruli have cellular crescents), sclerotic (> 50% glomeruli have fibrotic change), and mixed (crescentic/sclerotic) (Berden et al., 2010). These categories associated well with risk of ESRD (Fig. 160.2). The interstitium contains T- and B-cell infiltrates and T-cell tubulitis predicts a worse renal outcome (Berden et al., 2012).

Patients who develop ESKD should be considered for transplantation, which is a safe and effective option. Using United Network for Organ Sharing (UNOS) data from 1996 to 2007, 919 patients with ESKD secondary to GPA were identified. Adjusted outcomes for graft loss, death, or functional graft loss were better in those patients with GPA compared to other causes of ESKD (Shen et al., 2011). Relapses are uncommon following transplantation (Geetha et al., 2011). Similar to other patients with ESKD, survival is better in AAV patients who receive a kidney transplant compared to those who remain on dialysis (Merino et al., 2011). Outcome in those patients who remain on dialysis is similar to other causes of ESKD (Weidanz et al., 2007; Merino et al., 2011).

Quality of life

Despite resolution of disease activity, poor quality of life often persists in AAV patients with fatigue often described as the main contributor (Basu et al., 2010; Herlyn et al., 2010). In one study, 92% of 265 AAV patients ranked fatigue as their most significant symptom. Fatigue is not easily explained by disease factors (Faurschou et al., 2010). The mechanisms of fatigue are likely to be complex, with a balance between disease and psychosocial factors being important. Interestingly, despite many patients having significant organ damage due to disease, quality of life measures do not appear to correlate with the extent of damage.

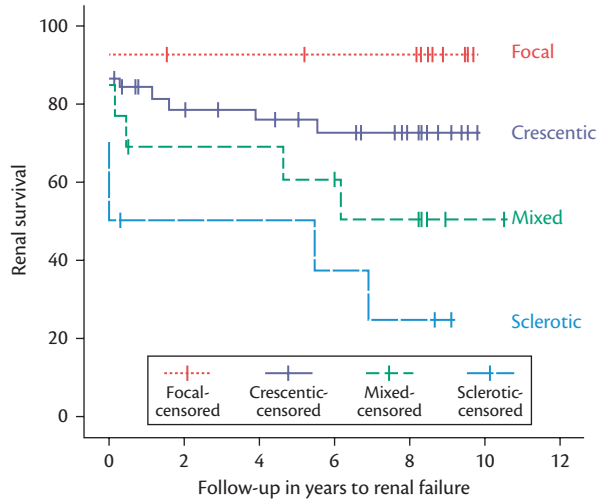

Fig. 160.2 A histological classification of ANCA-associated renal vasculitis and correlation with renal outcomes.
From Berden et al. (2010).

Future directions

Reducing diagnostic delay will have a major impact on outcomes but requires understanding of where patients present and subsequent referral pathways. Improved education of referring medical specialties and wider use of ANCA testing and urine analysis will facilitate earlier referral. Management in a sub-specialist vasculitis clinic with rapid access to associated specialities including ENT and respiratory medicine allows a coordinated approach to patient care and optimal assessment of disease extent and treatment response. Careful supervision of therapy, especially cyclophosphamide and high-dose glucocorticoids, has led to major reductions in severe adverse events over the last 20 years and late treatment-related toxicity. This is likely to be further improved by the availability of rituximab as an alternative to cyclophosphamide.

Glucocorticoids, delayed treatment response, and a high relapse rate are important components of the unmet need of current vasculitis therapies. Potential alternatives to glucocorticoids, such as IV immunoglobulin or TNF-α blockade, have proved impractical or ineffective. Targeted therapies at other cytokine, complement, or immune components may provide an opportunity to reduce glucocorticoids, such as the use of anti-TNF or IL-6 receptor antibodies. Rituximab has not led to a lower relapse rate than cyclophosphamide and there is uncertainty as to how to prevent relapse after rituximab induction. Other B-cell targeted therapies may be more effective or B-cell therapies may need to be continued during the remission phase.

The causes of impaired quality of life despite vasculitis remission are not understood, although subclinical disease activity is likely

in some patients. Other factors include ongoing treatment toxicity and irreversible damage. Newer therapies may impact on quality of life but there is no evidence for rituximab doing so currently.

References

Arulkumaran, N., Periselneris, N., Gaskin, G., *et al.* (2011). Interstitial lung disease and ANCA-associated vasculitis: a retrospective observational cohort study. *Rheumatology (Oxford)*, 50, 2035–43.

Bacon, P. A., Moots, R. J., Exley, A., *et al.* (1995). VITAL (Vasculitis Integrated Assessment Log) assessment of vasculitis. *Clin Exp Rheumatol*, 13, 275–8.

Bakoush, O., Segelmark, M., Torffvit, O., *et al.* (2006). Urine IgM excretion predicts outcome in ANCA-associated renal vasculitis. *Nephrol Dial Transplant*, 21, 1263–9.

Basu, N., Jones, G. T., Fluck, N., *et al.* (2010). Fatigue: a principal contributor to impaired quality of life in ANCA-associated vasculitis. *Rheumatology (Oxford)*, 49, 1383–90.

Berden, A. E., Ferrario, F., Hagen, E. C., *et al.* (2010). Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol*, 21, 1628–36.

Berden, A. E., Jones, R. B., Erasmus, D. D., *et al.* (2012). Tubular lesions predict renal outcome in antineutrophil cytoplasmic antibody-associated glomerulonephritis after rituximab therapy. *J Am Soc Nephrol*, 23(2), 313–21.

Bolton, W. K. and Couser, W. G. (1979). Intravenous pulse methylprednisolone therapy of acute crescentic rapidly progressive glomerulonephritis. *Am J Med*, 66, 495–502.

Booth, A. D., Almond, M. K., Burns, A., *et al.* (2003). Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. *Am J Kidney Dis*, 41, 776–84.

Booth, A., Harper, L., Hammad, T., *et al.* (2004a). Prospective study of TNFalpha blockade with infliximab in anti-neutrophil cytoplasmic antibody-associated systemic vasculitis. *J Am Soc Nephrol*, 1, 717–21.

Booth, A. D., Jayne, D. R., Kharbanda, R. K., *et al.* (2004b). Infliximab improves endothelial dysfunction in systemic vasculitis: a model of vascular inflammation. *Circulation*, 109, 1718–23.

Carrington, C. B. and Liebow, A. (1966). Limited forms of angiitis and granulomatosis of Wegener's type. *Am J Med*, 41, 497–527.

Cartin-Ceba, R., Golbin, J. M., Keogh, K. A., *et al.* (2012). Rituximab for remission induction and maintenance in refractory granulomatosis with polyangiitis (Wegener's): ten-year experience at a single center. *Arthritis Rheum*, 64, 3770–8.

Collagen Diseases and Hypersensitivity Panel (1957). Treatment of polyarteritis nodosa with cortisone: results after one year; report to the Medical Research Council by the Collagen Diseases and Hypersensitivity Panel. *Br Med J*, 1, 608–11.

Day, C. J., Howie, A. J., Nightingale, P., *et al.* (2010). Prediction of ESRD in pauci-immune necrotizing glomerulonephritis: quantitative histomorphometric assessment and serum creatinine. *Am J Kidney Dis*, 55, 250–8.

De Groot, K., Harper, L., Jayne, D. R., *et al.* (2009). Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial. *Ann Intern Med*, 150, 670–80.

De Groot, K., Rasmussen, N., Bacon, P. A., *et al.* (2005). Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum*, 52, 2461–9.

De Lind van Wijngaarden, R. A., Hauer, H. A., Wolterbeek, R., *et al.* (2006). Clinical and histologic determinants of renal outcome in ANCA-associated vasculitis: A prospective analysis of 100 patients with severe renal involvement. *J Am Soc Nephrol*, 17, 2264–74.

De Lind van Wijngaarden, R. A., Hauer, H. A., Wolterbeek, R., *et al.* (2007). Chances of renal recovery for dialysis-dependent ANCA-associated glomerulonephritis. *J Am Soc Nephrol*, 18, 2189–97.

De Sousa, E., Smith, R., Chaudhry, A., *et al.* (2012). Venous thromboembolism with concurrent pulmonary haemorrhage in systemic vasculitis. *Nephrol Dial Transplant*, 27(12), 4357–61.

Fauci, A. S., Wolff, S. M., and Johnson, J. S. (1971). Effect of cyclophosphamide upon the immune response in Wegener's granulomatosis. *N Engl J Med*, 285, 1493–6.

Faurschou, M., Sigaard, L., Bjorner, J. B., *et al.* (2010). Impaired health-related quality of life in patients treated for Wegener's granulomatosis. *J Rheumatol*, 37, 2081–5.

Faurschou, M., Sorensen, I. J., Mellemkjaer, L., *et al.* (2008). Malignancies in Wegener's granulomatosis: incidence and relation to cyclophosphamide therapy in a cohort of 293 patients. *J Rheumatol*, 35, 100–5.

Faurschou, M., Westman, K., Rasmussen, N., *et al.* (2012). Long-term outcome of a clinical trial comparing methotrexate to cyclophosphamide for remission induction of early systemic ANCA-associated vasculitis. *Arthritis Rheum*, 64(10), 3472–7.

Flossmann, O., Baslund, B., Bruchfeld, A., *et al.* (2009). Deoxyspergualin in relapsing and refractory Wegener's granulomatosis. *Ann Rheum Dis*, 68(7), 1125–30.

Flossmann, O., Berden, A., de Groot, K., *et al.* (2011). Long-term patient survival in ANCA-associated vasculitis. *Ann Rheum Dis*, 70, 488–94.

Freeley, S. J., Coughlan, A. M., Popat, R. J., *et al.* (2013). Granulocyte colony stimulating factor exacerbates antineutrophil cytoplasmic antibody vasculitis. *Ann Rheum Dis*, 72(6), 1053–8.

Geetha, D., Eirin, A., True, K., *et al.* (2011). Renal transplantation in antineutrophil cytoplasmic antibody-associated vasculitis: a multicenter experience. *Transplantation*, 91, 1370–5.

Gregersen, J. W., Kristensen, T., Krag, S. R., *et al.* (2012). Early plasma exchange improves outcome in PR3-ANCA-positive renal vasculitis. *Clin Exp Rheumatol*, 30, S39–47.

Guerry, M. J., Brogan, P., Bruce, I. N., *et al.* (2012). Recommendations for the use of rituximab in anti-neutrophil cytoplasm antibody-associated vasculitis. *Rheumatology (Oxford)*, 51(4), 634–43.

Guillevin, L., Cohen, P., Mahr, A., *et al.* (2003). Treatment of polyarteritis nodosa and microscopic polyangiitis with poor prognosis factors: a prospective trial comparing glucocorticoids and six or twelve cyclophosphamide pulses in sixty-five patients. *Arthritis Rheum*, 49, 93–100.

Guillevin, L., Mahr, A., Callard, P., *et al.* (2005). Hepatitis B virus-associated polyarteritis nodosa: clinical characteristics, outcome, and impact of treatment in 115 patients. *Medicine (Baltimore)*, 84, 313–22.

Guillevin, L., Pagnoux, C., Seror, R., *et al.* (2011). The Five-Factor Score revisited: assessment of prognoses of systemic necrotizing vasculitides based on the French Vasculitis Study Group (FVSG) cohort. *Medicine (Baltimore)*, 90, 19–27.

Harper, L., Morgan, M. D., Walsh, M., *et al.* (2012). Pulse versus daily oral cyclophosphamide for induction of remission in ANCA-associated vasculitis: long-term follow-up. *Ann Rheum Dis*, 71(6), 955–60.

Hauer, H. A., Bajema, I. M., van Houwelingen, H. C., *et al.* (2002). Renal histology in ANCA-associated vasculitis: differences between diagnostic and serologic subgroups. *Kidney Int*, 61, 80–9.

Heijl, C., Harper, L., Flossmann, O., *et al.* (2011). Incidence of malignancy in patients treated for antineutrophil cytoplasm antibody-associated vasculitis: follow-up data from European Vasculitis Study Group clinical trials. *Ann Rheum Dis*, 70, 1415–21.

Hellmich, B., Flossmann, O., Gross, W. L., *et al.* (2007). EULAR recommendations for conducting clinical studies and/or clinical trials in systemic vasculitis: focus on anti-neutrophil cytoplasm antibody-associated vasculitis. *Ann Rheum Dis*, 66, 605–17.

Herlyn, K., Hellmich, B., Seo, P., *et al.* (2010). Patient-reported outcome assessment in vasculitis may provide important data and a unique perspective. *Arthritis Care Res (Hoboken)*, 62, 1639–45.

Hervier, B., Pagnoux, C., Agard, C., *et al.* (2009). Pulmonary fibrosis associated with ANCA-positive vasculitides. Retrospective study of 12 cases and review of the literature. *Ann Rheum Dis*, 68, 404–7.

Hickman, R. A. and Gordon, C. (2011). Causes and management of infertility in systemic lupus erythematosus. *Rheumatology (Oxford)*, 50, 1551–8.

Hiemstra, T. F., Walsh, M., Mahr, A., *et al.* (2010). Mycophenolate mofetil vs azathioprine for remission maintenance in antineutrophil cytoplasmic antibody-associated vasculitis: A Randomized Controlled Trial. *JAMA*, 304(21), 2381–8.

Hoffman, G. S., Kerr, G. S., Leavitt, R. Y., *et al.* (1992). Wegener granulomatosis: an analysis of 158 patients. *Ann Intern Med*, 116, 488–98.

Hogan, S. L., Nachman, P. H., Wilkman, A. S., *et al.* (1996). Prognostic markers in patients with antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol*, 7, 23–32.

Hu, W., Liu, C., Xie, H., *et al.* (2008). Mycophenolate mofetil versus cyclophosphamide for inducing remission of ANCA vasculitis with moderate renal involvement. *Nephrol Dial Transplant*, 23, 1307–12.

Jayne, D. R., Chapel, H., Adu, D., *et al.* (2000). Intravenous immunoglobulin for ANCA-associated systemic vasculitis with persistent disease activity. *QJM*, 93, 433–9.

Jayne, D. R., Gaskin, G., Rasmussen, N., *et al.* (2007). Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. *J Am Soc Nephrol*, 18, 2180–8.

Jayne, D. R., Rasmussen, N., Andrassy, K., *et al.* (2003). A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med*, 349, 36–44.

Jones, R. B., Cohen Tervaert, J. W., Hauser, T., *et al.* (2010). Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med*, 363, 211–20.

Jones, R. B., Ferraro, A. J., Chaudhry, A. N., *et al.* (2009). A multicenter survey of rituximab therapy for refractory antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum*, 60, 2156–68.

Koldingsnes, W. and Nossent, H. (2002). Predictors of survival and organ damage in Wegener's granulomatosis. *Rheumatology (Oxford)*, 41, 572–81.

Kostianovsky, A., Hauser, T., Pagnoux, C., *et al.* (2012). Alveolar haemorrhage in ANCA-associated vasculitides: 80 patients' features and prognostic factors. *Clin Exp Rheumatol*, 30, S77–82.

Koukoulaki, M. and Jayne, D. R. (2006). Mycophenolate mofetil in anti-neutrophil cytoplasm antibodies-associated systemic vasculitis. *Nephron Clin Pract*, 102, c100–7.

Laudien, M., Lamprecht, P., Hedderich, J., *et al.* (2009). Olfactory dysfunction in Wegener's granulomatosis. *Rhinology*, 47, 254–9.

Leib, E. S., Restivo, C., and Paulus, H. E. (1979). Immunosuppressive and corticosteroid therapy of polyarteritis nodosa. *Am J Med*, 67, 941–7.

Little, M. A., Hassan, B., Jacques, S., *et al.* (2009). Renal transplantation in systemic vasculitis: when is it safe? *Nephrol Dial Transplant*, 24, 3219–25.

Little, M. A., Nightingale, P., Verburgh, C. A., *et al.* (2010). Early mortality in systemic vasculitis: relative contribution of adverse events and active vasculitis. *Ann Rheum Dis*, 69(6), 1036–43.

Lyons, P. A., Rayner, T. F., Trivedi, S., *et al.* (2012). Genetically distinct subsets within ANCA-Associated Vasculitis. *N Engl J Med*, 367, 214–23.

Mathieson, P. W., Cobbold, S. P., Hale, G., *et al.* (1990). Monoclonal-antibody therapy in systemic vasculitis. *N Engl J Med*, 323, 250–4.

McKinney EF, Lyons PA, Carr EJ, *et al.* (2010). A CD8(+) T cell transcription signature predicts prognosis in autoimmune disease. *Nat Med*, 16(5), 586–91.

Merino, J. L., Galeano, C., Espejo, B., *et al.* (2011). A retrospective study on outcome of microscopic polyangiitis in chronic renal replacement therapy. *Nephrol Dial Transplant*, 26, 1360–6.

Merkel, P. A., Lo, G. H., Holbrook, J. T., *et al.* (2005). Brief communication: high incidence of venous thrombotic events among patients with Wegener granulomatosis: the Wegener's Clinical Occurrence of Thrombosis (WeCLOT) Study. *Ann Intern Med*, 142, 620–6.

Metzler, C., Miehle, N., Manger, K., *et al.* Elevated relapse rate under oral methotrexate versus leflunomide for maintenance of remission in Wegener's granulomatosis. *Rheumatology (Oxford)*, 46, 1087–91.

Mukhtyar, C., Flossmann, O., Hellmich, B., *et al.* (2008). Outcomes from studies of antineutrophil cytoplasm antibody associated vasculitis: a systematic review by the European League Against Rheumatism systemic vasculitis task force. *Ann Rheum Dis*, 67, 1004–10.

Mukhtyar, C., Guillevin, L., Cid, M. C., *et al.* (2008). EULAR Recommendations for the management of primary small and medium vessel vasculitis. *Ann Rheum Dis*, 68, 310–17.

Mukhtyar, C., Guillevin, L., Cid, M. C., *et al.* (2008). EULAR recommendations for the management of large vessel vasculitis. *Ann Rheum Dis*, 68, 318–23.

Nachman, P. H., Hogan, S. L., Jennette, J. C., *et al.* (1996). Treatment response and relapse in antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol*, 7, 33–9.

Newman, W. G., Payne, K., Tricker, K., *et al.* (2011). A pragmatic randomized controlled trial of thiopurine methyltransferase genotyping prior to azathioprine treatment: the TARGET study. *Pharmacogenomics*, 12, 815–26.

Novack, S. N. and Pearson, C. M. (1971). Cyclophosphamide therapy in Wegener's granulomatosis. *N Engl J Med*, 284, 938–42.

Pagnoux, C., Hogan, S. L., Chin, H., *et al.* (2008). Predictors of treatment resistance and relapse in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis: Comparison of two independent cohorts. *Arthritis Rheum*, 58, 2908–18.

Pagnoux, C., Mahr, A., Hamidou, M. A., *et al.* (2008). Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. *N Engl J Med*, 359, 2790–803.

Pierrot-Deseilligny Despujol, C., Pouchot, J., Pagnoux, C., *et al.* (2010). Predictors at diagnosis of a first Wegener's granulomatosis relapse after obtaining complete remission. *Rheumatology (Oxford)*, 49(11), 2181–90.

Pillebout, E., Alberti, C., Guillevin, L., *et al.* (2010). Addition of cyclophosphamide to steroids provides no benefit compared with steroids alone in treating adult patients with severe Henoch Schonlein purpura. *Kidney Int*, 78, 495–502.

Rasmussen, N., Jayne, D., Abramovicz, D., *et al.* (1995). European therapeutic trials in ANCA-associated systemic vasculitis: disease scoring, consensus regimens and proposed clinical trials. *Clin Exp Immunol*, 101 Suppl 1, 29–34.

Reinhold-Keller, E., Beuge, N., Latza, U., *et al.* (2000). An interdisciplinary approach to the care of patients with Wegener's granulomatosis: long-term outcome in 155 patients. *Arthritis Rheum*, 43, 1021–32.

Sanders, J. S., Huitma, M. G., Kallenberg, C. G., *et al.* (2006). Prediction of relapses in PR3-ANCA-associated vasculitis by assessing responses of ANCA titres to treatment. *Rheumatology (Oxford)*, 45, 724–9.

Savage, C. O., Winearls, C. G., Jones, S., *et al.* (1987). Prospective study of radioimmunoassay for antibodies against neutrophil cytoplasm in diagnosis of systemic vasculitis. *Lancet*, 1, 1389–93.

Seror, R., Pagnoux, C., Ruivard, M., *et al.* (2010). Treatment strategies and outcome of induction-refractory Wegener's granulomatosis or microscopic polyangiitis: analysis of 32 patients with first-line induction-refractory disease in the WEGENT trial. *Ann Rheum Dis*, 69, 2125–30.

Shen, J., Gill, J., Shangguan, M., *et al.* (2011). Outcomes of renal transplantation in recipients with Wegener's granulomatosis. *Clin Transplant*, 25, 380–7.

Silva, F., Seo, P., Schroeder, D. R., *et al.* (2011). Solid malignancies among etanercept-treated patients with granulomatosis with polyangiitis (Wegener's): long-term followup of a multicenter longitudinal cohort. *Arthritis Rheum*, 63, 2495–503.

Silva, F., Specks, U., Kalra, S., *et al.* (2010). Mycophenolate mofetil for induction and maintenance of remission in microscopic polyangiitis with mild to moderate renal involvement—a prospective, open-label pilot trial. *Clin J Am Soc Nephrol*, 5(3), 445–53.

Smith, R. M., Jones, R. B., Guerry, M. J., *et al.* (2012). Rituximab for remission maintenance in relapsing ANCA-associated vasculitis. *Arthritis Rheum*, 64(11), 3760–9.

Stassen, P. M., Sanders, J. S., Kallenberg, C. G., *et al.* (2008). Influenza vaccination does not result in an increase in relapses in patients with ANCA-associated vasculitis. *Nephrol Dial Transplant*, 23, 654–8.

Stassen, P. M., Tervaert, J. W., and Stegeman, C. A. (2007). Induction of remission in active anti-neutrophil cytoplasmic antibody-associated vasculitis with mycophenolate mofetil in patients who cannot be treated with cyclophosphamide. *Ann Rheum Dis*, 66, 798–802.

Stegeman, C. A., Tervaert, J. W., Sluiter, W. J., *et al.* Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener granulomatosis. *Ann Intern Med*, 120, 12–17.

Stone, J. H., Merkel, P. A., Spiera, R., *et al.* (2010). Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med*, 363, 221–32.

Suppiah, R., Judge, A., Batra, R., *et al.* (2011). A model to predict cardiovascular events in patients with newly diagnosed Wegener's granulomatosis and microscopic polyangiitis. *Arthritis Care Res (Hoboken)*, 63, 588–96.

Tomasson, G., Grayson, P. C., Mahr, A. D., *et al.* (2012). Value of ANCA measurements during remission to predict a relapse of ANCA-associated vasculitis—a meta-analysis. *Rheumatology (Oxford)*, 51, 100–9.

Van Assen, S., Agmon-Levin, N., Elkayam, O., *et al.* (2011). EULAR recommendations for vaccination in adult patients with autoimmune inflammatory rheumatic diseases. *Ann Rheum Dis*, 70(3), 414–22.

Van der Woude, F. J., Rasmussen, N., Lobatto, S., *et al.* (1985). Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet*, 1, 425–9.

Venhoff, N., Effelsberg, N. M., Salzer, U., *et al.* (2012). Impact of rituximab on immunoglobulin concentrations and B cell numbers after cyclophosphamide treatment in patients with ANCA-associated vasculitides. *PLoS One*, 7, e37626.

Walsh, M., Catapano, F., Szpirt, W., *et al.* (2010). Plasma exchange for renal vasculitis and idiopathic rapidly progressive glomerulonephritis: a meta-analysis. *Am J Kidney Dis*, 57, 557–64.

Walsh, M., Chaudhry, A., and Jayne, D. (2008). Long-term follow-up of relapsing/refractory anti-neutrophil cytoplasm antibody associated vasculitis treated with the lymphocyte depleting antibody alemtuzumab (CAMPATH-1H). *Ann Rheum Dis*, 67, 1322–7.

Walsh, M., Flossmann, O., Berden, A., *et al.* (2012). Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum*, 64(2), 542–8

Walsh, M., Merkel, P. A., Mahr, A., *et al.* (2010). The effects of duration of glucocorticoid therapy on relapse rate in anti-neutrophil cytoplasm antibody associated vasculitis: A meta-analysis. *Arthritis Care Res (Hoboken)*, 62, 1166–73.

Walton, E. W. (1958). Giant-cell granuloma of the respiratory tract (Wegener's granulomatosis). *Br Med J*, 2, 265–70.

Wegener's Granulomatosis Etanercept Trial Research Group (2005). Etanercept plus standard therapy for Wegener's granulomatosis. *N Engl J Med*, 352, 351–61.

Weidanz, F., Day, C. J., Hewins, P., *et al.* (2007). Recurrences and infections during continuous immunosuppressive therapy after beginning dialysis in ANCA-associated vasculitis. *Am J Kidney Dis*, 50, 36–46.

Weidner, S., Geuss, S., Hafezi-Rachti, S., *et al.* (2004). ANCA-associated vasculitis with renal involvement: an outcome analysis. *Nephrol Dial Transplant*, 19, 1403–11.

CHAPTER 161

The patient with systemic lupus erythematosus: overview and pathogenesis

Johan van der Vlag and Jo H. M. Berden

Overview

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with various clinical manifestations. The hallmark of SLE is the presence of antibodies against nuclear constituents, like double-stranded (ds)DNA, histones, and nucleosomes (Mohan et al., 1993; Burlingame et al., 1994). Local deposition of antinuclear antibodies in complex with nuclear autoantigens induces serious inflammatory conditions that can affect several tissues and organs, including the kidney (Tsokos, 2011).

The levels of antinucleosome and anti-dsDNA antibodies seem to correlate with glomerulonephritis and these autoantibodies can often be detected years before the patient is diagnosed with SLE (Arbuckle et al., 2003). Apoptotic debris is present in the extracellular matrix and circulation of patients with SLE (Rumore and Steinman, 1990; Grootscholten et al., 2003; van Bavel et al., 2011) due to an aberrant process of apoptosis and/or insufficient clearance of apoptotic cells and apoptotic debris (Dieker et al., 2004; Munoz et al., 2008). The non-cleared apoptotic debris in patients with SLE may lead to activation of both the innate (myeloid and plasmacytoid dendritic cells) and adaptive (T and B cells) immune system (Fransen et al., 2010; Bouts et al., 2012). In addition to the activation by apoptotic debris and immune complexes, the immune system in SLE may be deregulated at the level of (a) presentation of self-peptides by antigen-presenting cells, (b) selection processes for both B and T cells, and (c) regulatory processes of B- and T-cell responses (Tsokos, 2011; Guerra et al., 2012; Liu and Davidson, 2012).

Lupus nephritis (see Chapter 162) may be classified in different classes based on histological findings in renal biopsies (Weening et al., 2004). The chromatin-containing immune complexes deposit in the capillary filter, most likely due to the interaction of chromatin with the polysaccharide heparan sulphate (van Bavel et al., 2008; O'Flynn et al., 2011; van der Vlag and Berden, 2011). A decreased renal expression of the endonuclease DNaseI further contributes to the glomerular persistence of chromatin and the development of glomerulonephritis (Zykova et al., 2010; Fismen et al., 2011; Seredkina and Rekvig, 2011).

Current treatment of lupus nephritis (see Chapter 163) is not specific and aims to reduce the inflammatory response with general immunosuppressive therapies. However, research has revealed novel potential therapeutic candidates at the level of dendritic cells, B cells, T cells, and cytokines (Tsokos, 2011; Liu and Davidson, 2012).

Pathogenesis: introduction

SLE particularly affects women during their fertile age. In the United States, the prevalence of SLE is about 50 per 100,000 persons. Autoantibodies against double-stranded (ds)DNA, nucleosomes, histones are characteristic for SLE (Mohan et al., 1993; Burlingame et al., 1994). Glomerulonephritis is one of the most serious clinical manifestations in SLE. The American College of Rheumatology (ACR) has designated 11 criteria for lupus covering the major clinical and laboratory features of the disease (see Table 161.1). A patient meeting four or more criteria out of 11 is diagnosed with SLE with 95% specificity and 85% sensitivity (Tan et al., 1982; Hochberg, 1997).

SLE has a multifactorial aetiology that is still not fully understood. The pathogenesis depends on a genetic predisposition, with contributing factors that may include infections, environmental factors, like sunlight and toxins, and hormonal factors (Tsokos, 2011). In the online database Online Mendelian Inheritance in Man ((OMIM) <http://www.ncbi.nlm.nih.gov/omim>) > 140 genes have been genetically associated in one way or another with SLE, while in PubMed (<http://www.ncbi.nlm.nih.gov/pubmed?db=pubmed>), the term lupus reveals > 66,000 scientific articles, which underscores the complexness of the pathogenesis of SLE.

The levels of antinucleosome and anti-dsDNA antibodies seem to correlate with glomerulonephritis and are already detectable before disease manifestations (Arbuckle et al., 2003). The main question that can be raised is how in SLE an autoimmune response is mounted against chromatin that normally is shielded from the immune system due to its location in the nucleus. Apoptotic material is present in the extracellular matrix and the circulation of patients with SLE (Rumore and Steinman, 1990; Grootscholten et al., 2003; van Bavel et al., 2011). The presence of apoptotic material may be the result of an aberrant process of apoptosis, either caused by an increased rate of apoptosis or apoptosis at the wrong moment or location (see Table 161.3 for an overview of factors) (Dieker et al., 2004; Munoz et al., 2008; Fransen et al., 2009). An insufficient clearance of apoptotic cells and debris provides an

Table 161.1 ACR revised classification criteria for systemic lupus erythematosus

Criterium	Definition
Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging
Photosensitivity	Skin rash as a result of unusual reaction to sunlight
Oral ulcers	Oral or nasopharyngeal ulceration
Arthritis	Non-erosive arthritis involving two or more peripheral joints
Serositis	A. Pleuritis or B. Pericarditis
Renal disorder	A. Persistent proteinuria (> 0.5 g/day) or B. Cellular casts
Neurologic disorder	A. Seizures, or B. Psychosis
Haematologic disorder	A. Haemolytic anaemia or B. Leucopenia or C. Lymphopenia or D. Thrombocytopenia
Immunologic disorder	A. Abnormal titre of antibodies to native DNA or B. Presence of antibody to Sm nuclear antigen or C. Positive finding of antiphospholipid antibodies
ANA	Abnormal titre of antinuclear antibodies

From Tan et al. (1982) and Hochberg (1997).

additional explanation for the persistent presence of apoptotic material in patients with SLE (see Table 161.4 for an overview) (Dieker et al., 2004; Fransen et al., 2009). The innate and adaptive immune system may thereby be activated in several ways: (a) apoptotic blebs and apoptotic chromatin, containing apoptosis-induced modifications, may lead to activation of myeloid dendritic cells (mDCs) via ligation of toll-like receptors (TLRs).These activated mDCs may present histone peptides in an immunogenic fashion to autoreactive T cells. Activated autoreactive T cells may activate autoreactive B cells specific for chromatin; (b) particular RNA-containing immune complexes may activate plasmacytoid dendritic cells (pDCs) via ligation of TLR7, leading to the production of type I interferons like IFN-α. Also chromatin-containing constituents of granulocytes, NETs (neutrophil extracellular traps) lead to activation of pDCs; (c) autoreactive B cells may be directly activated by apoptotic chromatin (Ronnblom et al., 2009; Fransen et al., 2010; Tsokos, 2011; Bouts et al., 2012; Liu and Davidson, 2012). This chapter will mainly focus on the first two pathways of immune activation. In addition to the three aforementioned pathways of immune activation, the immune system in SLE may be deregulated at the level of (a) presentation of self-peptides by antigen-presenting cells, (b) selection processes for both B and T cells, and (c) regulatory processes of B- and T-cell responses, including cytokines (Tsokos, 2011; Guerra et al., 2012; Liu and Davidson, 2012). See Table 161.2 for an overview of key factors implicated in the pathogenesis of SLE.

The pathogenesis of lupus nephritis is as complex as the aetiology of SLE, and several histological classifications have been proposed (Weening et al., 2004). The chromatin-containing immune complexes deposit in the capillary filter, most likely due to the interaction of positively charged histones in chromatin with the negatively charged polysaccharide heparan sulphate in the glomerular basement membrane and endothelial glycocalyx (van Bavel et al., 2011; van der Vlag and Berden, 2011; O'Flynn et al., 2011). A decreased renal expression of the endonuclease DNaseI further contributes to the development of glomerulonephritis (Zykova et al., 2010; Fismen et al., 2011; Seredkina and Rekvig, 2011). Current treatment of lupus nephritis is not specific and aims to reduce the inflammatory response with general immunosuppressive drugs. However, research in the last decade has revealed novel therapeutic targets at the level of dendritic cells, B cells, T cells, and cytokines (Tsokos, 2011; Kulkarni and Anders, 2012; Liu and Davidson, 2012).

Apoptosis and survival defects in systemic lupus erythematosus

Background of apoptosis and survival defects in SLE

Apoptosis is the process of programmed cell death and is involved in the formation, shaping, and maintenance of tissues and organs, including the regulation of the immune response by deletion of B and T cells. Apoptosis can be induced by intrinsic factors, such as DNA damage, and by extrinsic factors, such as, for example, the binding of Fas ligand to the Fas receptor. Apoptosis follows a cascade of signal transduction pathways that include caspases and endonucleases. Characteristic for apoptosis at the molecular level is the fragmentation of chromatin and at the cellular level the segregation of apoptotic blebs. Apoptotic blebs contain autoantigens targeted in SLE. In patients with SLE, apoptotic cells and immune complexes with nuclear autoantigens, such as nucleosomes, have been observed in several tissues, such as the germinal centre of the lymph nodes, the epidermis, the kidneys, and the circulation. A list of factors associated with apoptosis and SLE is provided in Table 161.3 (Fransen et al., 2009; Guerra et al., 2012). Data are derived from studies in both patients and mice, and some key factors will be briefly discussed.

Aberrant apoptosis induction and survival signals in SLE

One of the classical pathways which lead to the induction of apoptosis involves the Fas receptor (CD95; FasR) and Fas ligand (CD95L; FasL) couple. FasL is only expressed on immune cells, whereas FasR is expressed on non-immune cells as well. Mice deficient in FasR (lpr) or FasL (gld) show lymphoproliferation and development of SLE-like features. Mutations in the genes encoding FasR or FasL in humans lead to familial autoimmune lymphoproliferative syndrome, but not to SLE. In patients with juvenile onset of SLE an increased expression of FasR on T cells has been detected (Fransen et al., 2009).

Proteins of the Bcl-2 family are important regulators of apoptosis associated with SLE, and can be either anti-apoptotic (e.g. Bcl-2) or pro-apoptotic (e.g. Bim). Defects in Bim lead to the persistence of autoreactive B and T cells, the survival of antigen-presenting

Table 161.2 Pathogenic factors associated with SLE

Factor name	Abbreviation	Function
Major histocompatibility complex class II	HLA-DRB1	Binding and presenting antigenic peptides to T cells
Protein C-ets-1	ETS1	Transcription factor controlling differentiation and activation of B and T lymphocytes
Ikaros family zinc finger protein 1	IKZF1	Transcription factor controlling differentiation and activation of B and T lymphocytes
Protein tyrosine phosphatase, non-receptor type 22	PTPN22	Tyrosin phosphatase involved in B- and T-lymphocyte activation
Tumour necrosis factor (ligand) superfamily4	TNFSF4	Activation and adhesion of T cells
Programmed cell death-1	PDCD-1 (PD-1)	Negative regulator of activated T cells; required for development of regulatory T cells
Src homology 2 domain-containing transforming protein C1	Shc1 or p66Shc	Negative regulator of activated T cells
Signal transducer and activator of transcription4	STAT4	Transcription factor regulating T-cell development, T-cell activation, and cytokine production
cAMP-responsive element modulator	CREM	Transcription factor regulating IL-2 and IL-17 transcription in T cells
B-cell scaffold protein with ankyrin repeats	BANK1	Activation of B cells
B-lymphocyte kinase	BLK	Tyrosine kinase involved in B-cell activation
Toll-like receptor 2	TLR2	Receptor on phagocytes; binds nucleosome-associated high mobility group box protein 1 (HMGB1)
Toll-like receptors 7 and 9	TLR7 and -9	Endosomal receptor in phagocytes; binds single-stranded RNA/DNA, double-stranded DNA, respectively
Interleukin-1 receptor-associated kinase1	IRAK1	Kinase in downstream signalling pathways of the interleukin-1 receptor and toll-like receptors
Interferon regulatory factors 5 and 7	IRF5 and 7	Transcription factors regulating interferon alpha and beta production
Interferon regulatory factor 8	IRF8	Transcription factor controlling differentiation and activation of dendritic cell subsets
Interferon alpha	IFN-α	Cytokine with multiple effector functions (e.g. affecting T- and B-cell responses and survival)

Factors related to apoptosis and clearance are listed in Tables 161.3 and 161.4.

From Fransen et al. (2009), Guerra et al. (2012), and Tiffin et al. (2012).

cells, like DC, and are thereby facilitating the induction of autoimmunity. An increased expression of Bcl-2 on the other hand leads to apoptosis-resistant (autoreactive) lymphocytes (Fransen et al., 2009).

Integrin alpha-M, (ITGAM (CD11/CD18)) has been identified in genome-wide screens to be associated strongly with SLE. ITGAM functions in the regulation of apoptosis in neutrophils, but also in leucocyte adhesion and complement C3b binding. The precise function of ITGAM in neutrophil survival remains unclear (Nath et al., 2008).

A lack of survival signals also can lead to apoptosis. B-cell survival signals affected in SLE include the cytokine B-cell activating factor (BAFF), also known as BLyS, which binds to the BAFF-receptors, BAFFR, TACI, and BCMA. BAFF signalling is required for the maintenance of autoreactive B cells in the marginal zone, and an increased BAFF expression in mice results in the development of autoimmunity and SLE-like manifestations. Mouse models for lupus, like MRL/lpr and (NZBxNZW)F1, are characterized by a high expression of BAFF, whereas treatment of these lupus mice with a soluble receptor for BAFF, TACI, decreases mortality and inhibits the development of proteinuria (Cancro et al., 2009). In patients with SLE, elevated BAFF levels are present, which correlate with anti-dsDNA titres. In patients with SLE, a polymorphism in APRIL, related to the BAFF protein, has been identified as well. Recently a monoclonal antibody directed against BAFF, belimumab, was successfully used in patients with SLE (Dooley et al., 2013). Coronin-1A was identified in genome-wide screens to be associated with SLE and appeared to be a survival signal for T cells. Coronin-1A is associated with the actin cytoskeleton and a mutation in Coronin-1A is able to suppress autoimmunity and lupus nephritis in animal models for SLE (Haraldsson et al., 2008).

Neutrophil extracellular traps in SLE

Neutrophils may play an important role in the pathogenesis of SLE. Neutrophils can spill so-called neutrophil extracellular traps (NETs), which consist of their total chromatin and associated peptides with anti-microbial activity like LL37 and HNP. This process of NET formation is called NETosis and can be considered as a special case of apoptosis (Fuchs et al., 2007). The NETs are meant to capture microbes, but can also have adverse effects, as insufficient degradation of NETs is linked to SLE and lupus nephritis. Serum of patients with SLE contains anti-LL37 and anti-HNP antibodies, which correlate with anti-DNA antibody titres, indicating that these DNA-antimicrobial peptide complexes may serve as B cell autoantigens. These anti-LL37 and anti-HNP antibodies further facilitate

the process of NETosis (Bouts et al., 2012). In particular, the NETs are able to activate plasmacytoid dendritic cells (see 'The role of plasmacytoid dendritic cells and IFN-α in SLE') (Hakkim et al., 2010; Garcia-Romo et al., 2011; Kaplan, 2011; Lande et al., 2011; Bouts et al., 2012). Histones within NETs contain post-translational modifications including acetylation and methylation, which also may play a role in breaking the tolerance to NET-associated proteins; however, this mechanism has not yet been confirmed (Liu et al., 2012).

Apoptosis-induced autoantigen modifications in systemic lupus erythematosus

Autoantigens can be modified during apoptosis, whereby these modifications may facilitate breaching of tolerance. Autoantigens in SLE are prone to cleavage by caspases and endonucleases. Cleavage products of caspase and granzyme B, a protease that is activated in cytotoxic T-cell-induced apoptosis, appear to be more immunogenic than the intact molecules (Casciola-Rosen et al., 1999; Utz et al., 2000). In addition, autoantigens, including chromatin, may be post-translational modified through covalent addition of acetyl, phosphoryl, methyl, ubiquitin, citruline, ADP, or glutamine moieties. Autoantibodies against the aforementioned modifications are present in patients with SLE (Utz and Anderson, 1998; Doyle and Mamula, 2005; Munoz et al., 2008). Specific apoptosis-induced hyperacetylation patterns on histones H2A, H2B, and H4, as well as a specific methylation pattern on H3 have been associated with SLE (Dieker et al., 2007; van Bavel et al., 2009, 2011; Price et al., 2012). Plasma from patients with SLE and lupus mice reveal a higher reactivity with the identified acetylation and methylation patterns on histones, whereas hyperacetylated nucleosomes lead to maturation of DC from lupus-prone mice (see 'Dendritic cells in SLE') (Dieker et al., 2007).

In summary, several factors involved in the induction of apoptosis, anti-apoptotic factors, and survival factors are associated with SLE and glomerulonephritis. See Table 161.3 for an overview of factors related to apoptosis and survival defects in SLE. In addition, apoptosis-induced chromatin modifications and NETs have been linked to SLE.

Table 161.3 Factors associated with apoptosis and survival defects in SLE

Factor name	Abbreviation	Function
Integrin alpha M	ITGAM (CD11/CD18)	Regulator of apoptosis in neutrophils and binds to C3b-coated apoptotic cells
Fas receptor	FasR (CD95)	Inducer of apoptosis
Fas ligand	FasL (CD95L)	Inducer of apoptosis
BCL2-like 11 (apoptosis facilitator)	BCL2L11 or Bim	Inducer of apoptosis
B-cell lymphoma protein family	Bcl-2, BFl-1, Bcl-X_L	Inhibitor of apoptosis
Src homology 2 domain-containing transforming protein C1	Shc1 or p66Shc	Mediator of apoptosis in T cells
B-cell activating factor receptors	BAFFR TACI BCMA	Survival signal for B cells
B-cell activating factor	BAFF (BLyS)	Survival signal for B cells (binds to all BAFF receptors)
A proliferation-inducting ligand	APRIL	Survival signal for B cells (binds to TACI and BCMA)
Coronin-1A	Coro1a	Survival factor for T cells

From Fransen et al. (2009) and Guerra et al. (2012).

Clearance defects in systemic lupus erythematosus

Background of clearance defects in SLE

The mechanisms described in the previous paragraph explain how normally inaccessible autoantigens can become exposed to the immune system. In addition to an aberrant apoptosis, an impaired removal may lead to the accumulation of apoptotic cells and debris. Normally, apoptotic cells are swiftly removed through phagocytosis by professional phagocytes, such as macrophages, B cells and dendritic cells in a non-inflammatory or even anti-inflammatory manner. Depending on the context, phagocytosis of apoptotic cells may also result in a pro-inflammatory response, as will be detailed in later sections. The swift removal of apoptotic cells and debris normally prevents potentially harmful molecules being released. In the case of SLE the clearance capacity is apparently insufficient, and consequently apoptotic blebs will segregate from apoptotic cells. These apoptotic blebs contain clustered SLE-autoantigens, such as (modified) chromatin (Rosen and Casciola-Rosen, 1999). As discussed, in addition to apoptotic blebs, NETs can be released from neutrophils and be considered as apoptotic chromatin as well. The release of apoptotic chromatin autoantigens not only leads to the induction of autoimmunity but also to the formation of immune complexes. These immune complexes can deposit in the glomerular basement membrane thereby inciting a severe glomerulonephritis. So, both for the initiation of the immune response and the local inflammatory response, insufficient clearance may be a contributing factor in SLE. There is convincing evidence for clearance defects of apoptotic cells and debris in SLE. In fact it was clearly demonstrated that the clearance of apoptotic material by phagocytes is impaired in both lupus mice and patients (Herrmann et al., 1998; Licht et al., 2004). Downregulation of the expression of the endonuclease DNaseI in the kidney further contributes to the development of lupus nephritis (Seredkina and Rekvig, 2011). Factors associated with clearance defects in SLE are listed in Table 161.4 (Fransen et al., 2009; Guerra et al., 2012). Data are derived from studies in both patients and mice, and some key factors will be briefly discussed.

Aberrant recognition and opsonization of apoptotic cells in SLE

Cells undergoing apoptosis display 'come and get me' signals, like the lipid phosphatidylcholine (PC) or the protein thrombospondin, and 'eat me' signals, like the lipid phosphatidylserine (PS). These signals attract phagocytes and facilitate phagocytosis mediated by receptors on phagocytes. Bridging molecules, such as, for example, opsonins, serve as an additional link between the signals on the

surface of the apoptotic cell and the receptors on the phagocyte. Deficiencies in these components can lead to decreased clearance of apoptotic cells and to the development of SLE in humans and mice (Savill et al., 2002; Munoz et al., 2008; Fransen et al., 2009).

An important apoptotic cell signal for clearance is PS that is present at the outer cell membrane rapidly after the induction of apoptosis. PS is bound directly by receptors on the phagocyte or indirectly via bridging molecules or opsonins. The phagocytic cells express multiple receptors including the PS receptor, Milk fat globule-EGF factor 8 protein (MFGE8), complement factor C1q receptor, c-Mer proto-oncogene tyrosine kinase (MERTK), ITGAM, and macrophage receptor with collagenous structure (MARCO), which all enable binding of apoptotic cells. Macrophages deficient in these receptors show an impaired clearance of apoptotic cells and mice deficient in these receptors develop an increased number of antinuclear autoantibodies, indicating that defects in these receptors play a role in the development of SLE (Munoz et al., 2008; Fransen et al., 2009).

Bridging molecules and opsonins that play an important role in the clearance of apoptotic cells include complement C1q, pentraxins (PTX3), mannan-binding lectin (MBL), C-reactive protein (CRP), and serum amyloid P protein (SAP). Targeting of these molecules leads to autoimmunity and SLE-like features, including glomerulonephritis. The complement molecules C1q and MBL bind to late apoptotic cells in particular. C1q is an opsonin and required for uptake of degraded chromatin. Mice lacking C1q develop SLE, while in humans C1q deficiency always is associated with SLE. Polymorphisms in the MBL gene and low serum levels of MBL have been associated with SLE as well. CRP binding to apoptotic debris in conjunction with anti-CRP antibodies facilitates phagocytosis with a pro-inflammatory outcome. Binding of autoantibodies to apoptotic cells and particles is the classical example of opsonization, thereby facilitating phagocytosis via Fc-receptors on phagocytes (Sarmiento et al., 2007). Both the FcγRIIA and FcγRIIB receptor are genetically associated with the susceptibility to develop lupus nephritis (Munoz et al., 2008; Fransen et al., 2009).

DNaseI, the major endonuclease that fragments chromatin during apoptosis, is associated with SLE. DNaseI deficiency leads to the accumulation of non-fragmented apoptotic chromatin (Napirei et al., 2000). The 3′–5′ repair exonuclease (TREX1), another DNA metabolizing enzyme, is also associated with SLE (Rice et al., 2009). TREX1 facilitates the degradation of the reversed transcribed single-stranded DNA from retroviral elements. TREX1 deficiency leads to the accumulation of single-stranded DNA and the production of IFN-α by pDCs. Insufficient endonuclease activity also leads to the persistent presence of NETs (see 'Neutrophil extracellular traps in SLE').

Aberrant clearance of apoptotic cells in germinal centres in SLE

In addition to the systemic presence of apoptotic chromatin in the extracellular matrix and circulation, the accumulation of apoptotic debris in germinal centres of patients with SLE has been shown. In normal lymph nodes, apoptotic nuclei can be detected inside tangible body macrophages. Non-ingested apoptotic nuclei are often found outside these cells in SLE. Sometimes nuclear debris can be observed at the surfaces of follicular dendritic cells, which normally retain complement-opsonized immune complexes on their surfaces, thereby facilitating affinity maturation of B cells. Therefore, in SLE, clearance deficiency leads to the accumulation of apoptotic material on the follicular dendritic cells. The presence of not cleared apoptotic material at those sites may explain the loss of peripheral B-cell tolerance (Baumann et al., 2002; Munoz et al., 2008).

In summary, several factors involved in the attraction, recognition, and phagocytosis of apoptotic cells, and fragmentation of chromatin, are associated with SLE and glomerulonephritis. See Table 161.4 for an overview of factors related to clearance defects in SLE.

Table 161.4 Factors associated with clearance defects in SLE

Factor name	Abbreviation	Function
Integrin alpha M	ITGAM (CD11/CD18)	Binds to C3b-coated apoptotic cells and regulates apoptosis in neutrophils
Milk fat globule-EGF factor 8 protein	MFGE8	Binds to PS on apoptotic (B) cells and mediates uptake by phagocytes
c-mer proto-oncogene tyrosine kinase	MERTK	Mediates uptake of apoptotic cells by phagocytes
Macrophage receptor with collagenous structure	MARCO	Binds and clears apoptotic cells in the marginal zone of the spleen and in the thymus
Mannan-binding lectin	MBL	Binds to apoptotic cells and mediates uptake by phagocytes
Complement component 1, q subcomponent	C1q	Binds to apoptotic cells and mediates uptake by phagocytes
Pentraxin-related gene	PTX3	Binds to apoptotic cells and mediates uptake by phagocytes
Serum amyloid P-component	SAP	Binds to apoptotic cells and mediates uptake by phagocytes
C-reactive protein	CRP	Binds to apoptotic cells and mediates uptake by phagocytes
Fcγ receptor IIB	FCGRIIB (CD32)	Clears IgG immune complexes
Fcγ receptor IIA	FCGRIIA (CD32)	Clears IgG immune complexes
3′–5′ repair exonuclease 1	TREX1	Degrades single-stranded DNA reversed transcribed from retroviral elements
Deoxyribonuclease I	DNase1	Fragments chromatin

From Fransen et al. (2009) and Guerra et al. (2012).

Dendritic cells in systemic lupus erythematosus

The role of myeloid dendritic cells in SLE

Two main subsets of dendritic cells can be distinguished, mDCs and pDCs, which differ in their lineage and their expression of receptors, including the TLRs. In patients with SLE, soluble apoptotic chromatin is present in the circulation and the extracellular matrix (Rumore and Steinman, 1990; Grootscholten et al., 2003;

van Bavel et al., 2011). In addition, apoptotic blebs are present that contain clustered autoantigens, including apoptosis-induced chromatin (Rosen and Casciola-Rosen, 1999). The classical view is that macrophages can ingest apoptotic cells, blebs, and debris in an anti-inflammatory manner, which is characterized by the production of transforming growth factor beta and interleukin (IL)-10. In addition, dendritic cells encountering autoantigens without being activated will induce immunological tolerance. The balance between immunity and tolerance apparently is skewed towards autoimmunity in SLE. mDCs can be activated by apoptotic blebs and modified chromatin, and after ingestion mDCs present the modified histone peptides in a pro-inflammatory manner to T cells, thereby initiating an autoimmune response (Fransen et al., 2010). *In vitro* apoptotic bleb and apoptotic chromatin-matured mDCs show an increased expression of co-stimulatory molecules (CD86 and CD40) and increased secretion of proinflammatory cytokines (IL-1β, IL-6, and tumour necrosis factor alpha (TNF-α)) (Boule et al., 2004, 2012; Fransen et al., 2009a, 2009b). Chromatin of viable cells is less potent in activating mDCs. The effect of apoptotic blebs on mDCs is probably independent from TLR-3, -7, and -9. High-mobility group protein B1 (HMGB1) remains attached to apoptotic chromatin and appears crucial in activation of mDCs. TLR2 binds the HMGB1-complexed chromatin (Urbonaviciute et al., 2008). Presentation by activated mDCs of the ingested modified chromatin to autoreactive T cells may be the first step in breaking the immunological tolerance that may occur in patients with SLE. The secretion of IL-2, IFN-γ, and IL-17 in co-cultures of mDCs and T cells suggests T-cell polarization towards the T helper (Th)-1 and Th17 subtypes, while there is proof for a Th17 response in patients with SLE (Fransen et al., 2009b, 2010). IL-6 concentrations, produced by activated mDCs, are high in patients with SLE, which inhibit the development of regulatory T cells (T_{REG}) while it stimulates the development of Th17 cells, a feature linked to other autoimmune diseases as well. Th17 cells may activate autoreactive B cells and recruit inflammatory cells to specific organs (Garrett-Sinha et al., 2008). Activated autoreactive T cells, specific for apoptosis-modified histone peptides can also activate B cells which recognize either modified or unmodified parts of chromatin with their receptor, which results in the production of autoantibodies directed to modified and unmodified chromatin (DNA, histones, nucleosomes) via epitope spreading. After formation of autoantibodies, immune complexes with circulating chromatin are formed that can activate mDCs, thereby creating an amplification loop in the immune response against apoptotic chromatin.

The role of plasmacytoid dendritic cells and IFN-α in SLE

Plasmacytoid DC express TLR7 and TLR9, whereas mDCs from human blood do not abundantly express these specific TLRs. pDCs are not so big eaters as mDCs, and, for example, do not ingest apoptotic blebs. Immune complexes composed of nucleic acids and antibodies specific for these nucleic acids or associated proteins are ingested by pDCs via FcγRIIA. Subsequently, via ligation of TLR7 and TLR9 the pDCs are activated, thereby initiating the production of type I IFNs, with IFN-α as key cytokine. In patients with SLE, a type I IFN response is frequently observed, indicating that pDC activation is a central event in the pathogenesis of SLE. IFN-α has a broad range of effector functions, which include mDC maturation, B-cell activation, T-cell activation, and enhancing NETosis, thereby amplifying the autoimmune response against chromatin (Ronnblom et al., 2009). In addition to nucleic acid-containing immune complexes, the previously introduced NETs also trigger pDCs to produce IFN-α (Garcia-Romo et al., 2011; Kaplan, 2011; Lande et al., 2011; Bouts et al., 2012).

Renal pathogenicity of antichromatin autoantibodies in lupus nephritis

The deposition of immune complexes containing anti-chromatin and chromatin in basement membranes is typical for a type III immunological reaction of which SLE is the prototype disease. Essentially two models exist to explain the pathogenicity in lupus nephritis of anti-chromatin antibodies in general, and anti-dsDNA antibodies in particular. In the first model, cross-reactivity of anti-dsDNA antibodies with intrinsic glomerular structures such as laminin, type IV collagen, or α-actinin initiates the inflammatory reaction. In the second model, chromatin mediates the binding of anti-chromatin antibodies to glomerular structure such as heparan sulphates that are present in the glomerular basement membrane and in the glomerular endothelial glycocalyx (van Bavel et al., 2008; van der Vlag and Berden, 2011). Most evidence is in favour of the second model. The presence of chromatin bound to anti-dsDNA antibodies used in research could serve as an explanation for apparent cross-reactive binding, since removal of chromatin from these antibody preparations leads to loss of the cross-reactivity (Termaat et al., 1990). Now it is clear that *in vivo* bound anti-chromatin antibodies in lupus nephritis only co-localize with deposited chromatin, and not with intrinsic glomerular structures (Kalaaji et al., 2006a, 2006b; van Bavel et al., 2008). The accumulation of large chromatin fragments in the kidney is enhanced and facilitated by a specific and local shutdown of DNaseI (Zykova et al., 2010; Fismen et al., 2011; Seredkina and Rekvig, 2011).

Treatment of lupus nephritis

For the treatment of SLE four primary classes of drugs can be distinguished: non-steroidal anti-inflammatory compounds (NSAIDs), antimalarials (e.g. hydroxychloroquine), corticosteroids (e.g. prednisone), and cytotoxic/immunosuppressive agents (e.g. azathioprine, mycophenolate mofetil, ciclosporin, and cyclophosphamide). Current therapies in general are not lupus specific and aim to suppress the autoimmune response. However, novel therapies for lupus are emerging; they include antibodies against B cells (e.g. rituximab, ocrelizumab, and epratuzumab) and compounds affecting the survival of B cells (e.g. belimumab and belatacept that bind BlyS and APRIL). Antibodies neutralizing key cytokines in the pathogenesis of SLE (e.g. for TNF-α, IFN-α, or IL-6), complement inhibitors, and blockers for the costimulatory interactions between B and T cells, and APC and T cells (e.g. for CD40L, CTLA4-Ig) are currently available or developed (Tsokos, 2011; Kulkarni and Anders, 2012; Liu and Davidson, 2012). Although promising, these novel therapies are not specific for treatment of SLE and lupus nephritis. The holy grail in treatment of SLE is to induce tolerance against chromatin. Histone-peptides can induce tolerance in mouse models for lupus, which is characterized by autoantigen-specific expansion of regulatory T cells and contraction of Th17 cells (Kang et al., 2005, 2007). A leading example of a lupus-specific tolerizing peptide is Lupuzor™ (P140 peptide), which

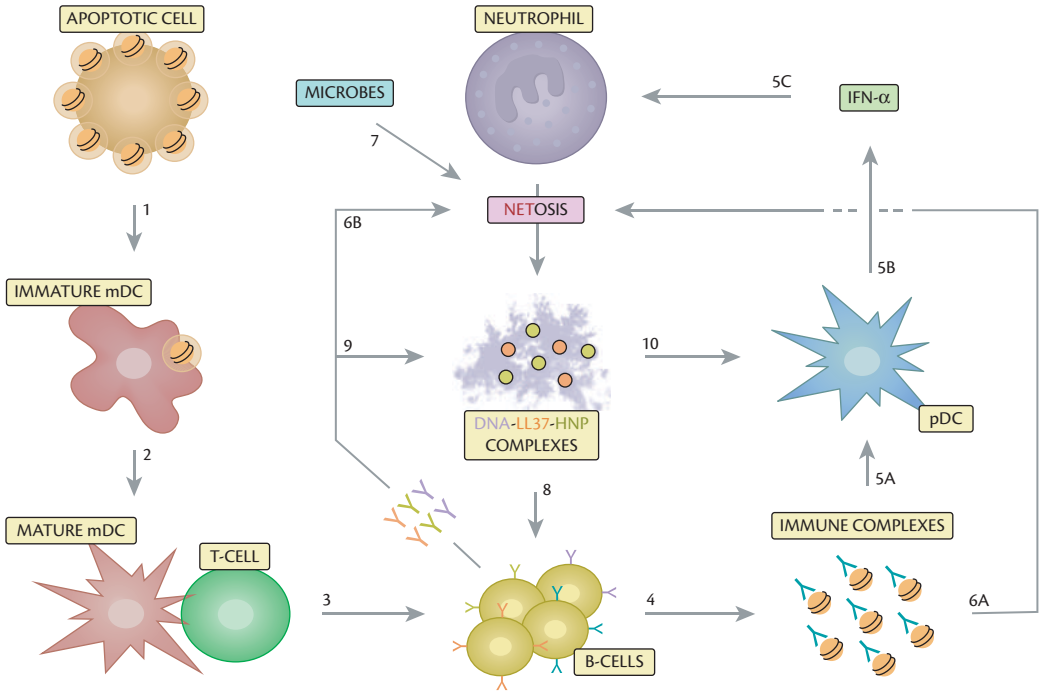

Fig. 161.1 Integrated model for the pathogenesis of SLE. (1) Apoptotic blebs and chromatin is ingested by immature myeloid dendritic cells (mDC), which, thereby, (2) are matured and present (apoptosis-modified) chromatin in their MHC to autoreactive T cells. (3) Activated autoreactive T cells assist autoreactive B cells to produce autoantibodies directed against chromatin. (4) Immune complexes between autoantibodies and chromatin are formed. (5A) Immune complexes are ingested by plasmacytoid dendritic cells (pDCs), which, thereby are activated and (5B) start to produce type I interferons, including IFN-α. (5C) IFN-α primes neutrophils, and (6A, 6B) autoantibodies against chromatin, and NET-associated proteins LL37 and HNP, induce NETosis, which is normally triggered by microbes (7). (8) Chromatin and NET-associated proteins (LL37, HNP) spewed into the extracellular space function as autoantigens for the B cell, which leads to anti-LL37, anti-HNP, and anti-chromatin autoantibodies that may form immune complexes with NET (9), thereby facilitating their uptake by pDC (10). This establishes a loop between pDC and neutrophils that chronifies and/or exacerbates the autoimmune response and the inflammatory condition in SLE.
Adapted from Bouts et al. (2012).

is in the clinical trial phase. Lupuzor™ is based on a peptide (peptide 140) derived from the U1-70K snRNP protein, which contains a residue (serine 140) that is phosphorylated during apoptosis. In lupus mouse models, administration of the P140 peptide reduces mortality and proteinuria. In a phase 2a study, Lupuzor™ led to a significant reduction of anti-DNA antibodies and improvement of the SLE disease activity index (SLEDAI) (Baumann et al., 2002; Dieker et al., 2008; Muller et al., 2008).

Concluding remarks

Despite the research conducted and the large number of papers published, we do not yet fully understand the aetiology of SLE. During the last decades, important factors and processes have been identified that may contribute to the development of autoimmunity and lupus nephritis. In Fig. 161.1, an integrated hypothesis for the initiation and amplifying processes in SLE is depicted. Central processes in the pathogenesis of SLE seem to be apoptosis, including NETosis, and the clearance of apoptotic material and NETs. Apoptosis-induced chromatin modifications, present in soluble chromatin, apoptotic blebs, and immune complexes may lead to the activation of mDCs and pDCs. Activated mDCs may initiate a mixed Th1/Th17 response, whereas activated pDCs produce type I IFNs, with IFN-α as a key player, affecting B and T cells, which chronifies the disease. Novel therapeutics should interfere with these central processes and aim to induce tolerance.

References

Arbuckle, M. R., McClain, M. T., Rubertone, M. V., *et al.* 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med*, 349, 1526–33.

Baumann, I., Kolowos, W., Voll, R. E., *et al.* (2002). Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. *Arthritis Rheum*, 46, 191–201.

Boule, M. W., Broughton, C., Mackay, F., *et al.* (2004). Toll-like receptor 9-dependent and -independent dendritic cell activation by chromatin-immunoglobulin G complexes. *J Exp Med*, 199, 1631–40.

Bouts, Y. M., Wolthuis, D. F. G. J., Dirkx, M. F. M., *et al.* J. (2012). Apoptosis and NET formation in the pathogenesis of SLE. *Autoimmunity*, 45, 597–601.

Burlingame, R. W., Boey, M. L., Starkebaum, G., *et al.* (1994). The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus-erythematosus. *J Clin Invest*, 94, 184–92.

Cancro, M. P., D'Cruz, D. P., and Khamashta, M. A. (2009). The role of B lymphocyte stimulator (BLyS) in systemic lupus erythematosus. *J Clin Invest*, 119, 1066–73.

Casciola-Rosen, L., Andrade, F., Ulanet, D., *et al.* (1999). Cleavage by granzyme B is strongly predictive of autoantigen status: Implications for initiation of autoimmunity. *J Exp Med*, 190, 815–25.

Dieker, J., Cisterna, B., Monneaux, F., *et al.* (2008). Apoptosis-linked changes in the phosphorylation status and subcellular localization of the spliceosomalautoantigen U1-70K. *Cell Death Differ*, 15, 793–804.

Dieker, J. W., Fransen, J. H., Van Bavel, C. C., *et al.* (2007). Apoptosis-induced acetylation of histones is pathogenic in systemic lupus erythematosus. *Arthritis Rheumat*, 56, 1921–33.

Dieker, J. W. C., Van Der Vlag, J., and Berden, J. H. M. (2004). Deranged removal of apoptotic cells: its role in the genesis of lupus. *Nephrol Dial Transplant*, 19, 282–5.

Dooley, M. A., Houssiau, F., Aranow, C., *et al.* (2013). Effect of belimumab treatment on renal outcomes: results from the phase 3 belimumab clinical trials in patients with SLE. *Lupus*, 22, 63–72.

Doyle, H. A. and Mamula, M. J. (2005). Posttranslational modifications of self-antigens. *Autoimmunity*, 1050, 1–9.

Fismen, S., Mortensen, E. S., and Rekvig, O. P. (2011). Nuclease deficiencies promote end-stage lupus nephritis but not nephritogenic autoimmunity in (NZBxNZW) F1 mice. *Immunol Cell Biol*, 89, 90–9.

Fransen, J. H., Hilbrands, L. B., Jacobs, C. W., *et al.* (2009a). Both early and late apoptotic blebs are taken up by DC and induce IL-6 production. *Autoimmunity*, 42, 325–7.

Fransen, J. H., Hilbrands, L. B., Ruben, J., *et al.* (2009b). Mouse dendritic cells matured by ingestion of apoptotic blebs induce T cells to produce interleukin-17. *Arthritis Rheumat*, 60, 2304–13.

Fransen, J. H., Van Der Vlag, J., Ruben, J., *et al.* (2010). The role of dendritic cells in the pathogenesis of systemic lupus erythematosus. *Arthritis Res Ther*, 12(2), 207.

Fransen, J. H., Hilbrands, L. B., Koeter, C.M., *et al.* (2009). The role of apoptosis and removal of apoptotic cells in the genesis of systemic lupus erythematosus. *Arch Med Sci*, 5, S466–77.

Fuchs, T. A., Abed, U., Goosmann, C., *et al.* (2007). Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol*, 176, 231–41.

Garcia-Romo, G. S., Caielli, S., Vega, B., *et al.* (2011). Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Transl Med*, 3(73), 73ra20.

Garrett-Sinha, L. A., John, S., and Gaffen, S. L. (2008). IL-17 and the Th17 lineage in systemic lupus erythematosus. *Curr Opin Rheumatol*, 20, 519–25.

Grootscholten, C., Van Bruggen, M. C. J., Van Der Pijl, J. W., *et al.* (2003). Deposition of nucleosomal antigens (histones and DNA) in the epidermal basement membrane in human lupus nephritis. *Arthritis Rheumat*, 48, 1355–62.

Guerra, S. G., Vyse, T. J., and Cunninghame Graham, D. S. (2012). The genetics of lupus: a functional perspective. *Arthritis Res Ther*, 14, 211.

Hakkim, A., Furnrohr, B. G., Amann, K., *et al.* (2010). Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc Natl Acad Sci U S A*, 107, 9813–8.

Haraldsson, M. K., Louis-Dit-Sully, C. A., Lawson, B. R., *et al.* (2008). The lupus-related Lmb3 locus contains a disease-suppressing Coronin-1A gene mutation. Immunity, 28, 40–51.

Herrmann, M., Voll, R. E., Zoller, O. M., *et al.* (1998). Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. *Arthritis Rheumat*, 41, 1241–50.

Hochberg, M. C. (1997). Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheumat*, 40, 1725–5.

Kalaaji, M., Mortensen, E., Jorgensen, L., *et al.* (2006a). Nephritogenic lupus antibodies recognize glomerular basement membrane-associated chromatin fragments released from apoptotic intraglomerular cells. *Am J Pathol*, 168, 1779–92.

Kalaaji, M., Sturfelt, G., Mjelle, J. E., *et al.* (2006b). Critical comparative analyses of anti-alpha-actinin and glomerulus-bound antibodies in human and murine lupus nephritis. *Arthritis Rheumat*, 54, 914–26.

Kang, H. K., Liu, M., and Datta, S. K. (2007). Low-dose peptide tolerance therapy of lupus generates plasmacytoid dendritic cells that cause expansion of autoantigen-specific regulatory T cells and contraction of inflammatory Th17 cells. *J Immunol*, 178, 7849–58.

Kang, H. K., Michaels, M. A., Berner, B. R., *et al.* (2005). Very low-dose tolerance with nucleosomal peptides controls lupus and induces potent regulatory T cell subsets. *J Immunol*, 174, 3247–55.

Kaplan, M. J. (2011). Neutrophils in the pathogenesis and manifestations of SLE. *Nat Rev Rheumatol*, 7, 691–9.

Kulkarni, O. P. and Anders, H. J. (2012). Lupus nephritis. How latest insights into its pathogenesis promote novel therapies. *Curr Opin Rheumatol*, 24, 457–65.

Lande, R., Ganguly, D., Facchinetti, V., *et al.* (2011). Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci Transl Med*, 3(73), 73ra19.

Licht, R., Dieker, J. W. C., Jacobs, C. W. M., *et al.* (2004). Decreased phagocytosis of apoptotic cells in diseased SLE mice. *J Autoimmun*, 22, 139–45.

Liu, C. L., Tangsombatvisit, S., Rosenberg, J. M., *et al.* (2012). Specific post-translational histone modifications of neutrophil extracellular traps as immunogens and potential targets of lupus autoantibodies. *Arthritis Res Ther*, 14(1), R25.

Liu, Z. and Davidson, A. (2012). Taming lupus-a new understanding of pathogenesis is leading to clinical advances. *Nat Med*, 18, 871–82.

Mohan, C., Adams, S., Stanik, V., *et al.* (1993). Nucleosome—a major immunogen for pathogenic autoantibody-inducing T-cells of lupus. *J Exp Med*, 177, 1367–81.

Muller, S., Monneaux, F., Schall, N., *et al.* (2008). Spliceosomal peptide P140 for immunotherapy of systemic lupus erythematosus results of an early phase II clinical trial. *Arthritis Rheumat*, 58, 3873–83.

Munoz, L. E., Van Bavel, C., Franz, S., *et al.* (2008). Apoptosis in the pathogenesis of systemic lupus erythematosus. *Lupus*, 17, 371–5.

Napirei, M., Karsunky, H., Zevnik, B., *et al.* (2000). Features of systemic lupus erythematosus in Dnase1-deficient mice. *Nat Genet*, 25, 177–81.

Nath, S. K., Han, S. Z., Kim-Howard, X., *et al.* (2008). A nonsynonymous functional variant in integrin-alpha M (encoded by ITGAM) is associated with systemic lupus erythematosus. *Nat Genet*, 40, 152–4.

O'flynn, J., Flierman, R., Van Der Pol, P., *et al.* (2011). Nucleosomes and C1q bound to glomerular endothelial cells serve as targets for autoantibodies and determine complement activation. *Mol Immunol*, 49, 75–83.

Price, J. V., Tangsombatvisit, S., Xu, G. Y., *et al.* (2012). On silico peptide microarrays for high-resolution mapping of antibody epitopes and diverse protein-protein interactions. *Nat Med*, 18, 1434-+.

Rice, G. I., Bond, J., Asipu, A., *et al.* J. (2009). Mutations involved in Aicardi-Goutieres syndrome implicate SAMHD1 as regulator of the innate immune response. *Nature Genet*, 41, 829–89.

Ronnblom, L., Alm, G. V., and Eloranta, M. L. (2009). Type I interferon and lupus. Curr *Opin Rheumatol*, 21, 471–7.

Rosen, A. and Casciola-Rosen, L. (1999). Autoantigens as substrates for apoptotic proteases: implications for the pathogenesis of systemic autoimmune disease. *Cell Death Differ*, 6, 6–12.

Rumore, P. M. and Steinman, C. R. (1990). Endogenous circulating DNA in systemic lupus erythematosus. Occurrence as multimeric complexes bound to histone. *J Clin Invest*, 86, 69–74.

Sarmiento, L. F., Munoz, L. E., Chirinos, P., *et al.* (2007). Opsonization by anti-dsDNA antibodies of apoptotic cells in systemic lupus erythematosus. *Autoimmunity*, 40, 337–9.

Savill, J., Dransfield, I., Gregory, C., *et al.* (2002). A blast from the past: clearance of apoptotic cells regulates immune responses. *Nat Rev Immunol*, 2, 965–75.

Seredkina, N. and Rekvig, O. P. (2011). Acquired loss of renal nuclease activity is restricted to DNasel and is an organ-selective feature in murine lupus nephritis. *Am J Pathol*, 179, 1120–8.

Tan, E. M., Cohen, A. S., Fries, J. F., *et al.* (1982). Special article—the 1982 revised criteria for the classification of systemic lupus-erythematosus. *Arthritis Rheumat*, 25, 1271–7.

Termaat, R. M., Brinkman, K., Vangompel, F., *et al.* (1990). Cross-reactivity of monoclonal anti-DNA antibodies with heparan-sulfate is mediated via bound DNA histone complexes. *J Autoimmun*, 3, 531–45.

Tiffin, N. A., Adeyemo, A., and Okpechi, I. (2012). A diverse array of genetic factors contribute to the pathogenesis of systemic lupus erythematosus. *Orphanet J Rare Dis*, 8, 2.

Tsokos, G. C. (2011). Mechanisms of disease systemic lupus erythematosus. *N Engl J Med*, 365, 2110–21.

Urbonaviciute, V., Furnrohr, B. G., Meister, S., *et al.* (2008). Induction of inflammatory and immune responses by HMGB1-nucleosome complexes: implications for the pathogenesis of SLE. *J Exp Med*, 205, 3007–18.

Utz, P. J. and Anderson, P. (1998). Posttranslational protein modifications, apoptosis, and the bypass of tolerance to autoantigens. *Arthritis Rheumat*, 41, 1152–60.

Utz, P. J., Gensler, T. J., and Anderson, P. (2000). Death, autoantigen modifications, and tolerance. *Arthritis Res*, 2, 101–14.

Van Bavel, C. C., Dieker, J. W., Kroeze, Y., *et al.* (2011). Apoptosis-induced histone H3 methylation is targeted by autoantibodies in systemic lupus erythematosus. *Ann Rheumat Dis*, 70, 201–7.

Van Bavel, C. C., Dieker, J., Muller, S., *et al.* (2009). Apoptosis-associated acetylation on histone H2B is an epitope for lupus autoantibodies. *Mol Immunol*, 47, 511–6.

Van Bavel, C. C., Fenton, K. A., Rekvig, O. P., *et al.* (2008). Glomerular targets of nephritogenic autoantibodies in systemic lupus erythematosus. *Arthritis Rheumat*, 58, 1892–9.

Van Der Vlag, J. and Berden, J. H. M. (2011). Lupus nephritis: role of antinucleosome autoantibodies. *Semin Nephrol*, 31, 376–89.

Weening, J. J., D'Agati, V. D., Schwartz et al. (2004). The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int*, 65, 521–30.

Zykova, S. N., Tveita, A. A., and Rekvig, O. P. (2010). Renal dnase1 enzyme activity and protein expression is selectively shut down in murine and human membranoproliferative lupus nephritis. *PLoS One*, 5(8), pii: e12096.

CHAPTER 162

The patient with systemic lupus erythematosus: clinical features, investigations, and diagnosis

Marie Condon, Philippa Dodd, and Liz Lightstone

Introduction

Systemic lupus erythematosus (SLE) is a chronic, relapsing, inflammatory, often febrile multisystemic disorder, characterized by involvement of the skin, joints, visceral organs, and serosal membranes. Symptoms and manifestations vary widely over an unpredictable relapsing and remitting course.

Clinical features: symptoms and physical signs

The presentation of SLE is highly variable, ranging from mild forms to severe disease requiring hospitalization. Most commonly it manifests as a combination of constitutional symptoms with cutaneous, musculoskeletal, mild haematological, and serological involvement (Von Feldt, 1995); however, it can be more severe, and even life-threatening, when renal, haematological, or central nervous system (CNS) disease predominates. There is a tendency for the disease pattern present at the time of onset to prevail during subsequent exacerbations (Fessler and Boumpas, 1995).

Constitutional symptoms

Non-specific fatigue, fever, weakness, anorexia, and unintentional weight loss are the most common symptoms of new presentations and recurrent disease flares. Fatigue is often multifactorial in origin and may be a symptom of an affective disorder (Wang et al., 1998; Wolfe et al., 2009) but is perhaps the hardest symptom to evaluate and the impact of which on daily life is often underestimated. Fever is also common but non-specific with multiple aetiologies including active disease, infection, and drug reaction; careful exclusion of infection should be sought prior to attributing fever to active SLE, particularly in the immune-compromised patient.

Mucocutaneous manifestations

Mucocutaneous manifestations are present in > 80% of patients with SLE. Cutaneous manifestations have been categorized according to the Gilliam classification of lupus erythematosus (LE) skin disease (Sontheimer, 1997). Skin disease falls into three categories—acute cutaneous (ACLE), subacute cutaneous (SCLE), and chronic cutaneous lupus erythematosus (CCLE). Localized ACLE is widely recognized as a classic photosensitive erythematous rash affecting the malar eminences and nasal bridge with characteristic sparing of the nasolabial folds—the 'butterfly' rash of SLE. SCLE lesions exist as two morphological variants—annular and papulo-squamous types and are also photosensitive. CCLE, more commonly known as classic discoid LE, occurs in up to 20–25% of patients with SLE (Pistiner et al., 1991). It presents as erythematous papular or macular lesions, which develop into discoid plaques. Lesions are most commonly found on the face, ears, neck, and extensor forearms (Rothfield et al., 2006).

Musculoskeletal manifestations

Musculoskeletal manifestations occur in > 95% of patients with SLE (Rothfield et al., 2006). Arthralgias are typically migratory affecting any joint, usually for less than a 24-hour period. Any joint may be affected but most commonly those of the small joints of the hands and fingers and the knees. Elbows, shoulders, hips, and sacroiliac joints are less commonly affected. Although SLE arthropathy is generally considered to be non-deforming, approximately 15–50% of patients will suffer deformity akin to that of rheumatoid arthritis (van Vugt et al., 1998; Grossman, 2009). Erosions are rarely notable on plain radiographs and require more sensitive imaging such as magnetic resonance imaging (MRI) for detection (Ostendorf et al., 2003). Anti-CCP antibody positivity correlates strongly with erosive arthropathy (Chan et al., 2008) and deforming arthropathies correlate with anti-Ro and/or -La antibodies (Franceschini et al., 1994). Femoral osteonecrosis is not uncommon especially in those who have received high-dose and/or long-term corticosteroids. There is an association between loss of bone mineral density and corticosteroid use; however, even those who have never received corticosteroids are also at risk.

Ophthalmological manifestations

Ocular disease can occur via immune complex deposition, antibody-related mechanisms, vasculitis, and thrombosis. The most common ocular disorder occurring in SLE is keratoconjunctivitis sicca due to lacrimal gland dysfunction leading to a reduction in the aqueous component of tears causing a sensation of 'gritty' eyes. Complications include corneal ulceration and infection. Episcleritis can occur and is usually self-limiting. Scleritis is

much more serious and may threaten sight, especially in association with anterior uveitis and may require systemic therapy (Sivaraj et al., 2007). Retinal vasculitis can also be present; it may threaten sight when there is occlusion of retinal arterioles and consequential infarction. Large vessel occlusions from thrombotic vascular disease typically occur in association with antiphospholipid or anticardiolipin antibody (Ravelli et al., 1993).

Haematological manifestations

Anaemia is a common clinical finding occurring in most throughout the disease course. The most common aetiology is anaemia of chronic disease but iron deficiency anaemia, autoimmune haemolytic anaemia (AIHA), anaemia of chronic renal failure, and drug-induced myelotoxicity can all contribute significantly. Aetiological mechanisms often coexist. Leucopenia is common, often reflective of disease activity but may be pharmacologically induced (Keeling and Isenberg, 1993). Lymphopenia, affecting particularly suppressor T cells, is associated with several clinical and immunological manifestations of SLE, most often observed during periods of higher disease activity (Nossent and Swaak, 1991). Neutropenia most often occurs as a result of drug induced marrow suppression but may be the result of antineutrophil antibodies, hypersplenism, or primary bone marrow failure such as that observed in the haemophagocytic syndrome (Martinez-Banos et al., 2006). Thrombocytopenia occurs frequently, almost invariably due to antibody-mediated platelet destruction but severe bleeding is unusual. Rarely, platelet consumption may occur in conjunction with microangiopathic haemolysis.

Non-coronary cardiac manifestations

Cardiac disease is categorized as pericardial, valvular, myocardial, and coronary in origin. Historically it was severe and life-threatening but now, although common, is often mild and asymptomatic. Pericardial disease, acute or chronic, is documented in up to 54% of patients but often asymptomatic (Doria et al., 2005). Valvular cardiac disease is either functional, due to anaemia, fever, and tachycardias, or more commonly structural. Thickening of valve leaflets, caused by valvulitis then healing with fibrosis, is the most common lesion. The mitral valve, including prolapse which occurs more frequently in SLE, is more commonly affected then the aortic valve. Disease is generally asymptomatic and detected clinically by a mild to moderate murmur (Roldan et al., 1996). Libman–Sacks endocarditis is referred to as a more extreme phenotype affecting mitral, aortic, and/or tricuspid valves. Approximately 10% have lesions detectable by transthoracic echocardiography but 40% by transoesophageal echocardiography (Roldan et al., 2008). The valvular lesions consist of immune complexes, mononuclear cells, fibrin, and platelet thrombi and healing of the lesions causes fibrosis, scarring, and calcification with valve damage and regurgitation (Moder et al., 1999). Although typically asymptomatic, systemic embolization and increased risk of secondary infective endocarditis occur. Myocarditis manifests clinically as a resting tachycardia, cardiomegaly and ST-T wave abnormalities on electrocardiography. Symptoms and signs of cardiac failure, arrhythmias, and conduction abnormalities may accompany cardiomegaly (Moder et al., 1999). Myocardial biopsy may be indicated to differentiate active myocarditis of SLE from other causes. Conduction abnormalities can occur due to fibrosis.

Vascular manifestations

Coronary artery disease (CAD) is a major cause of premature morbidity and mortality with a prevalence of 6–10% in patients suffering with SLE. Atherosclerosis, coronary arteritis, thrombotic events (with or without antiphospholipid antibodies (aPL)), vasospasm, and embolization have all been implicated in the development of CAD. Accelerated atherosclerosis occurs due to higher rates of traditional risk factors seen in SLE patients and underlying systemic vascular inflammation (Rahman et al., 2000) coupled with the side effects of glucocorticoid therapy. Control of secondary risk factors and disease control, using steroid-sparing agents where possible, underpins treatment strategies. These risk factors also contribute to a higher risk and prevalence of cerebrovascular disease. Raynaud's phenomenon and livedo reticularis are also typical features of SLE. Increased rates of vascular thrombosis, with or without aPL positivity, may occur and can result in recurrent thromboembolic disease.

Pulmonary manifestations

Pulmonary involvement may affect the airways, parenchyma, vasculature, the pleura, and/or the diaphragm and constitutes the presenting problem in 4–5% of cases (Bencivelli et al., 1992) with complaints of pleurisy, coughing, and shortness of breath. Pleural disease is the most common thoracic manifestation of SLE (Crestani, 2005; Torre and Harari, 2011). Effusions are typically bilateral, with immune complex deposition thought to underlie the pathogenesis. Respiratory tract infection is common due to the effect of glucocorticoids, other immunosuppressive medications, and the underlying immunological dysfunction characteristic of SLE particularly the hypocomplementaemia. Acute disease, manifesting as acute lupus pneumonitis (prevalence of 1–4%) and diffuse pulmonary haemorrhage (prevalence of 0.5–5.7%, associated with renal disease (Torre and Harari, 2011)), although uncommon are potentially life-threatening. Interstitial lung disease affects up to one-third of patients with variable degrees of severity. Pulmonary embolism has a significantly greater prevalence in SLE sufferers, with the presence of aPL leading to a further sixfold increase in risk (Wahl et al., 1998).

Gastrointestinal manifestations

Gastrointestinal manifestations affect approximately half of patients reflecting the effect of lupus or medications used in its treatment (Ebert and Hagspiel, 2011). Dysphagia is a common complaint usually resulting from hypomotility attributable to ischaemia or vasculitis affecting Auerbach's plexus, or inflammatory changes within the oesophageal musculature (Castrucci et al., 1990). Other causes include strictures, reflux disease, candidiasis, ulceration and medication-induced oesophagitis. The use of NSAIDs and corticosteroids significantly increases the risk of peptic ulcer disease (Griffin and Smalley, 1995) thus prophylactic therapy should be employed (Sultan et al., 1999). When there is a complaint of abdominal pain, infection (such as cytomegalovirus), peritonitis, peptic ulcer disease, mesenteric vasculitis with infarction and perforation, and pancreatitis should be considered. Hepatic abnormalities are common, often limited to abnormal biochemistry but are potentially progressive particularly in the context of coexistent liver disease (Matsumoto et al., 1992; Chowdhary et al., 2008). Abnormal liver chemistry reflects disease activity, medication use, or other coincidental liver diseases. Jaundice is a rarity, usually

indicative of haemolysis rather than hepatic impairment. If SLE is causative, appropriate treatment of SLE should result in normalization of liver chemistry. Coexisting autoimmune liver diseases are rare but should be established via the presence or absence of appropriate antibodies and biopsy.

Neuropsychiatric manifestations

Neuropsychiatric manifestations are protean occurring in 10–80% of patients either prior to diagnosis or during the disease course (Wong et al., 1991; Futrell et al., 1992; Sibley et al., 1992; Joseph et al., 2007). Common neurological syndromes include cognitive dysfunction; seizure activity; peripheral neuropathy; mononeuritis multiplex (Vina et al., 2005); cranial neuropathies (Bertsias et al., 2010); stroke (Mikdashi et al., 2007); transient ischaemic attacks and recurrent small vessel infarcts, most commonly associated with aPL (Toubi et al., 1995); CNS vasculitis occurs in the context of active lupus, manifesting as fever, headache, and acute confusional states prior to the onset of psychosis, seizures, and coma; organic psychosis attributable to CNS lupus but also glucocorticoid therapy (Ward and Studenski, 1991); and depression and anxiety (Schneebaum et al., 1991). Other neuropsychiatric manifestations include transverse myelitis, movement disorders (Joseph et al., 2007), and septic or aseptic meningitis. SLE has also been associated with reversible posterior leucoencephalopathy syndrome (also known as PRES) (Streck Ade et al., 2012).

Lupus nephritis

Histologically, almost all patients have evidence of lupus-associated renal disease despite the absence of clinical signs of renal disease. Up to 75% of patients have been reported to manifest clinical evidence of nephropathy at some stage during the disease (Cervera et al., 1993, 2003; Seligman et al., 2002; Kasitanon et al., 2006). Nephropathy is often an early manifestation usually within 6 months and 3 years (Nossent et al., 1990; Seligman et al., 2002) following diagnosis and nearly 50% within the first year (Seshan and Jennette, 2009) although a rise in plasma creatinine is uncommon within the first few years following diagnosis. Age, gender, and ethnicity are significantly influential in the time course for its development (Seligman et al., 2002; Patel et al., 2006). Lupus nephritis (LN) is much more common in patients not of European white descent and is often more severe.

Renal involvement is usually first diagnosed on the basis of urine dipstick testing for microscopic haematuria and proteinuria—hence dipstick testing should be a routine part of the clinical evaluation of patients at each visit. The most common clinical manifestation of LN is proteinuria, frequently nephrotic range with development of nephrotic syndrome. Patients can present with 'frothy urine' or oedema in the context of nephrotic syndrome (peripheral or periorbital classically) but are often asymptomatic. Microscopic haematuria is highly prevalent affecting approximately 80% of patients but rarely appears in isolation. Tubular abnormalities are common due to immune complex deposition in tubular basement membranes and the effects of interstitial nephritis. In a registry of patients from Spain, renal impairment (glomerular filtration rate (GFR) < 60 mL/min) was present in approximately 40% of patients at diagnosis (Vozmediano et al., 2012) with progression to end-stage renal failure in approximately 17% overall; however, this is significantly higher in the black population (Adler et al., 2006). A small proportion of patients, 1–2%, will suffer acute kidney injury attributable to thrombotic microangiopathy (TMA), often in association with aPL, acute interstitial nephritis, renal vascular thrombosis, or widespread glomerular crescentic disease. Other features associated with LN include the presence of red cell and/or granular urinary casts, hypertension, and hyperkalaemia in the context of renal tubular acidosis (Cameron, 1999).

The prevalence of each feature is variable and non-uniform across and within each class of nephritis thus renal pathology cannot be predicted with any real degree of certainty from the clinical picture; however, there are certain clinical features that in combination are more likely to occur with the class of LN. For a full histological description of the classes of LN please go to the 'Investigations' section.

- Classes I and II: mild haematuria and/or proteinuria (<1 g/24 hours); mildly reduced creatinine clearance in < 15%.
- Class III: 50% have active urinary sediment; up to 30% have nephrotic range proteinuria; renal insufficiency in 10–25%; dsDNA antibodies and reduced serum complement in > 50%.
- Class IV: proteinuria universal and up to 50% nephrotic; active urinary sediment in most, 30–40% hypertensive, > 50% have renal insufficiency, dsDNA antibodies and/or reduced serum complement in up to 85%.
- Class V: virtually all patients have proteinuria in pure class V; up to 70% nephrotic; 50% haematuria; renal insufficiency uncommon; anti-dsDNA antibodies are less frequently elevated and ANA antibodies may be undetectable; complement levels may be normal or slightly reduced. Can manifest prior to and in the absence of any other systemic or immunological manifestations of SLE.

Similarly, clinical outcomes are highly variable, dependent upon the class of disease and extent of activity and chronicity pertaining to each lesion as well as the effectiveness of and adherence to a particular treatment regimen. With this in mind, the duration of untreated renal disease is also of paramount importance.

Investigations

Investigating SLE depends to an extent on the presentation of the individual. However, a number of haematological, biochemical, and immunological investigations provide useful diagnostic information, either for the disease itself or in context of organ system involvement, and should be performed routinely.

Laboratory tests

Full blood count, renal, bone, and lipid profiles, liver function tests, inflammatory markers, urinalysis, and urine protein:creatinine ratio on a spot urine should be performed routinely to assess the presence of target organ disease (Bertsias et al., 2012). Complement assay is very useful as consumption, particularly of C3, is commonly associated with disease activity. Creatinine kinase measurement is useful in cases of suspected myositis as are the Coombs test and reticulocyte count to exclude autoimmune haemolysis in the presence of anaemia.

Antibodies

Clinical research to date has identified a large number of auto-antibodies associated with SLE and its various manifestations.

- Antinuclear antibody (ANA) is one of the best diagnostic tests and in high titre is almost invariably associated with SLE and therefore should be measured in all suspected cases. Whilst its sensitivity is high, specificity is low, therefore results need to be correlated appropriately with clinical features and other immune parameters.
- Anti-double-stranded DNA (dsDNA) antibodies are highly specific for a diagnosis of SLE. Anti-dsDNA antibodies are documented to have a specificity of 75–100%, sensitivity of 66–95%, and positive predictive value of 89–100%. Titres can correlate strongly with disease activity and consequently are used as a monitor of disease. High titres of immunoglobulin (Ig)-G anti-dsDNA antibodies have been associated with active glomerulonephritis both in serum and in the form of glomerular deposits. The importance of anti-histone antibodies has also been documented in the context of LN.
- Anti-Sm antibodies are also highly specific for SLE, 55–100% but with a sensitivity of only 25%. They are detectable in the absence of anti-dsDNA antibodies but tend to remain stable rather than fluctuate with disease activity.
- Anti-ribonucleoprotein (RNP) antibodies bind antigens that are related to Sm antigens and are found in up to 69% of patients with SLE and are associated with severe disease and Raynaud's phenomenon.
- Anti-Ro/SSA antibodies are found in 10–60% of patients with SLE depending on the method of assay and have been associated with lymphopenia, SCLE, neonatal lupus, and Sjögren syndrome.
- Anti-La/SSE antibodies are positive in 10–35% of patients with SLE and usually occur in combination with anti-Ro antibodies—they are associated with neonatal lupus and Sjögren syndrome.
- Anti-ribosomal P protein antibodies are reported in 10–50% of patients, depending on the population studied. Their diagnostic value is reported to be limited due to low sensitivity but when present, these antibodies have 80% specificity for neuropsychiatric manifestations.
- The various aPL are present in an estimated 30% of patients with SLE. They have been proven to act as an independent risk factor for premature death in patients with SLE therefore they are routinely measured. The antiphospholipid syndrome is discussed in Chapter 164.
- Anti-C1q antibodies have relatively fair sensitivity and specificity in the diagnosis of LN, suggesting that the presence of anti-C1q antibodies may be a valuable adjunct for predicting LN and assessing renal activity (Yin et al., 2012); however, currently they are not generally in clinical use.

From the perspective of monitoring disease activity, the combination of falling C3 and rising anti-dsDNA antibody titres would cause concern that a flare is imminent—however, the presence of the changes should prompt closer follow-up and warning the patient to report symptoms early rather than pre-emptive additional immunosuppression.

Imaging

No imaging technique is diagnostic of SLE but may be useful in investigating specific symptoms or laboratory abnormalities. Imaging techniques include plain X-rays usually of painful joints and the chest, ultrasonography of kidneys or liver following detection of biochemical abnormalities, echocardiography to assess potential pericardial or valvular disease, computed tomography scans of the chest or abdomen in suspected cases of interstitial lung disease or pancreatitis respectively, MRI scans of the brain in cases of focal neurological deficits or cognitive impairment, and contrast angiography in cases of suspected pulmonary embolism or mesenteric and limb ischaemia.

Renal biopsy

As per Kidney Disease: Improving Global Outcomes (KDIGO) guidance from 2012 (KDIGO GN Work Group, 2012), the presence of LN should be considered in any lupus patient with impaired kidney function, proteinuria, hypertension, or an active urine sediment. An active sediment includes haematuria, especially acanthocytes suggestive of glomerular bleeding, leucocyturia in the absence of infection, and red and white blood cell casts. LN must be confirmed by kidney biopsy. The histologic findings provide the basis for treatment recommendations for LN (Radhakrishnan and Cattran, 2012). The importance of this lies in the variable clinical manifestations of each class of nephritis and the limitation of current biomarkers of renal involvement to differentiate milder disease from more aggressive forms and those with a less favourable prognosis or identify the degree of chronic damage. Initial biopsy is also a valuable tool in determining therapeutic regimens (Hill et al., 2001). It may confirm a diagnosis of SLE in patients who have uncertain diagnoses such as no convincing serological evidence or the presence of proteinuria with bland urine (Adu et al., 1983; Christopher-Stine et al., 2007). It should be performed as soon as possible, as early diagnosis and treatment have been proven to benefit outcome. Repeat renal biopsy is generally performed in those who fail to respond to treatment, those who relapse following induction of remission, and those who exhibit progressive disease with new or worsening proteinuria and active urinary sediment, or a falling GFR/rising creatinine.

Patterns of glomerular injury

Glomerular immune complex deposition is the hallmark of LN, with their location, number, and ability to provoke an inflammatory response correlating strongly with the severity and pattern of injury (Fries et al., 1988). Three distinct patterns of glomerular injury have been identified—mesangial, endothelial, and epithelial.

- *Mesangial* immune complex deposits result in variable degrees of mesangial cell proliferation and matrix accumulation. Histologically this manifests as mesangial hypercellularity and increased matrix deposition (Fig. 162.1) and clinically as subnephrotic proteinuria with microscopic haematuria usually in the context of well-preserved or minimally impaired renal function.
- *Subendothelial* patterns of deposits are associated with an exudative component to lesions with accumulation of leucocytes, endothelial cell injury, and endocapillary proliferation with destruction of capillary walls, immune complex deposition, and variable mesangial proliferation (Fig. 162.2). The mesangium and subendothelial spaces are in direct contact with one another lying proximal to the glomerular basement membrane (GBM), therefore having access to the vascular space. Persistent accumulation of deposits within these spaces leads to more severe

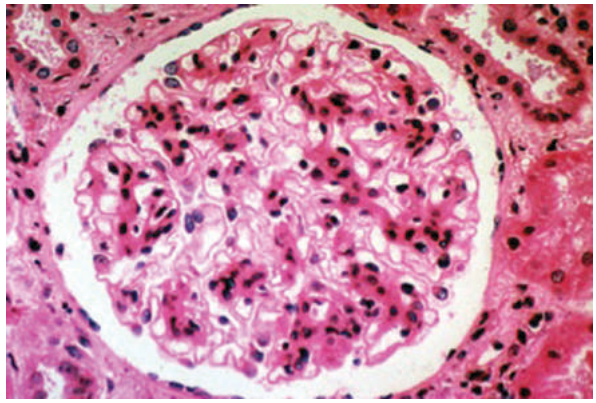

Fig. 162.1 LM of glomerulus with mesangial hypercellularity.

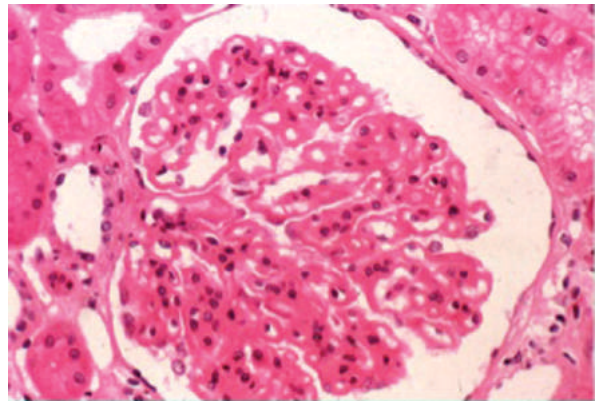

Fig. 162.3 LM of glomerulus with GBM swelling (membranous) lesion.

injury as the deposits activate complement, generate chemoattractants, and recruit neutrophils and mononuclear cells from the circulating vascular supply. Histologically this results in a proliferative glomerulonephritis, focal or diffuse, presenting clinically with an active urinary sediment, significant, often nephrotic range, proteinuria, and declining renal function.

- *Subepithelial* immune complex deposits cause injury via antibody- and complement-mediated cytotoxicity directed at the podocyte. A non-exudative, non-proliferative lesion of the capillary wall ultimately results (Fig. 162.3). The absence of inflammatory cell infiltration due to the separating effect of the GBM confines damage to the glomerular epithelial cells. Histologically this manifests as a membranous nephropathy and clinically with a bland urinary sediment and significant, often nephrotic range, proteinuria (Weening et al., 2004).

A variety of differing morphological lesions have been described as a result of these patterns of injury and it should be remembered that lesions often coexist, overlapping in any given combination, thus leading to a complex clinical expression of disease.

Immunohistochemistry

Typically 'full house' that is, deposition of IgG, IgA, IgM, C1q, C3, C4. Presence of C1q and C4 indicates classical pathway activation and should always raise the suspicion of SLE as should the finding of immune deposits in multiple sites, that is, mesangial, subendothelial, and subepithelial. Fig. 162.4 demonstrates IgG staining on immunofluorescence,

Classification of lupus nephritis

The first publication attempting to classify LN was in 1964 by Pollak et al. titled 'The natural history of the renal manifestations of systemic lupus erythematosus'. Using a classification based solely on light microscopy, the authors subdivided LN into normal, lupus glomerulitis, active lupus glomerulonephritis, and membranous lupus glomerulonephritis and they also scored active and inactive lesions (Fig. 162.5C). They followed 87 patients from 7 months to 8 years. They demonstrated a good prognostic value with only 2/40 patients with normal biopsies, glomerulitis, or membranous dying of renal failure, compared with 26/47 with active lupus glomerulonephritis dying of renal failure (Pollak et al., 1964). In 1974, the World Health Organization (WHO) first divided LN into five different histological patterns (Fig. 162.5A). This was modified in 1982 to include subdivisions relating to activity and chronicity of lesions at which time classification of class VI lesions was also introduced. Further modification in 1995 placed emphasis upon the significance of segmental necrosis, reflecting an increasing understanding of the pathogenesis of LN. The WHO classification was revised in 2004 under sponsorship of the International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) in order

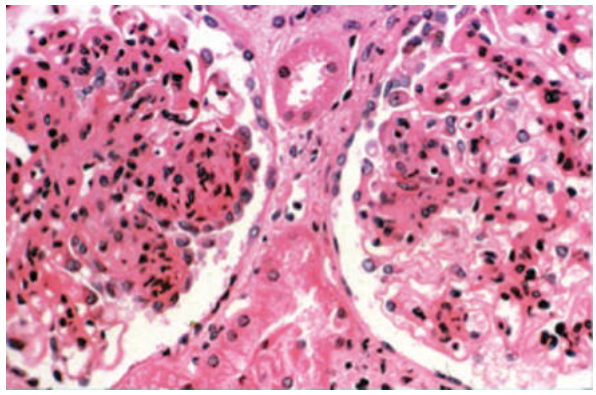

Fig. 162.2 LM of glomerulus with subendothelial cell swelling and endocapillary proliferation.

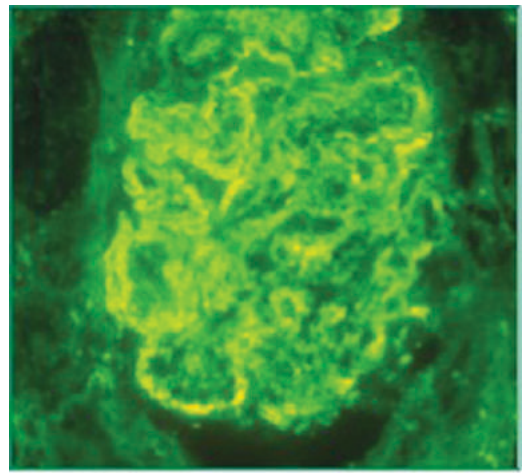

Fig. 162.4 IgG deposition within the glomerulus.

(A)

Class I	Normal glomeruli (by light microscopy, immunofluorescence, and electron microscopy)
Class II	Purely mesangial disease
	a. Normocellular mesangium by light microscopy but mesangial deposits by immunofluorescence or electron microscopy
	b. Mesangial hypercellularity with mesangial deposits by immunofluorescence or electron microscopy
Class III	Focal proliferative glomerulonephritis (<50%)
Class IV	Diffuse proliferative glomerulonephritis (≥50%)
Class V	Membranous glomerulonephritis

From Appel et al. (1978).

(B)

Class I	**Minimal mesangial lupus nephritis**
	Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence
Class II	**Mesangial proliferative lupus nephritis**
	Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits
	May be a few isolated subepithelial or subendothelial deposits visible by immunofluorescence or electron microscopy, but not by light microscopy
Class III	**Focal lupus nephritis**
	Active or inactive focal, segmental or global endo- or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations
Class III (A)	Active lesions: focal proliferative lupus nephritis
Class III (A/C)	Active and chronic lesions: focal proliferative and sclerosing lupus nephritis
Class III (C)	Chronic inactive lesions with glomerular scars: focal sclerosing lupus nephritis
Class IV	**Diffuse lupus nephritis**
	Active or inactive diffuse, segmental or global endo- or extracapillary glomerulonephritis involving ≥ 50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) lupus nephritis when ≥ 50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) lupus nephritis when ≥ 50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits but with little or no glomerular proliferation
Class IV-S (A)	Active lesions: diffuse segmental proliferative lupus nephritis
Class IV-G (A)	Active lesions: diffuse global proliferative lupus nephritis
Class IV-S (A/C)	Active and chronic lesions: diffuse segmental proliferative and sclerosing lupus nephritis
	Active and chronic lesions: diffuse global proliferative and sclerosing lupus nephritis
Class IV-S (C)	Chronic inactive lesions with scars: diffuse segmental sclerosing lupus nephritis
Class IV-G (C)	Chronic inactive lesions with scars: diffuse global sclerosing lupus nephritis
Class V	**Membranous lupus nephritis**
	Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations
	Class V lupus nephritis may occur in combination with class III or IV in which case both will be diagnosed
	Class V lupus nephritis show advanced sclerosis
Class VI	**Advanced sclerosis lupus nephritis**
	≥90% of glomeruli globally sclerosed without residual activity

From Weening et al. (2004).

(C)

Active lesions
Endocapillary hypercellularity with or without leukocyte infiltration and with substantial luminal reduction
Karyorrhexis
Fibrinoid necrosis
Rupture of glomerular basement membrane
Crescents, cellular or fibrocellular
Subendothelial deposits identifiable by light microscopy (wire loops)
Intraluminal immune aggregates (hyaline thrombi)
Chronic lesions
Glomerular sclerosis (segmental, global)
Fibrous adhesions
Fibrous crescents

From Weening et al. (2004).

Fig. 162.5 Original WHO and ISN/RPS classifications. (A) Original 1974 WHO classification of LN. (B) 2003 ISN/RPS classification of LN. (C) Active and chronic glomerular lesions.

to accommodate the developments in clinicopathologic and pathogenetic insights that had accumulated since the 1995 modification to the WHO classification (Weening et al., 2004) (Fig. 162.5B). The ISN/RPS classification preserves the simplicity of the original classification system, is based exclusively upon glomerular pathology, adding qualitative and quantitative differences between class III and IV lesions and emphasizing the importance of standardization of definitions, clinically relevant lesions, and uniform reporting between centres (Seshan and Jennette, 2009). An alternative 'flow chart approach' to the ISN/RPS classification was published in 2006, demonstrating the clarity and simplicity of the classification (Elliot et al., 2006) (Fig. 162.6).

Class I lupus nephritis

Class I lesions exhibit mesangial accumulation of immune complexes identifiable by immunofluorescence (IF) and electron microscopy (EM) but with no alterations on light microscopy (LM) (Fig. 162.7) and is termed minimal mesangial LN. EM may also demonstrate tubuloreticular inclusions within endothelial cells (Fig. 162.8); these can be present in any class of LN. EM examination aids in confirming the presence of features identified by LM and IF and identifies subtle features essential for diagnosis. Although WHO classifications included specimens with normal glomeruli by LM, EM, and IF, the ISN/RSP classification stipulates that lack of abnormalities using all three examination methods no longer qualifies as class I disease.

Class II lupus nephritis

Class II lesions are characterized by any degree of mesangial hypercellularity (Fig. 162.9: three or more mesangial cells per mesangial region in 3-micron thick section) in association with mesangial immune complex deposition, thus termed mesangial

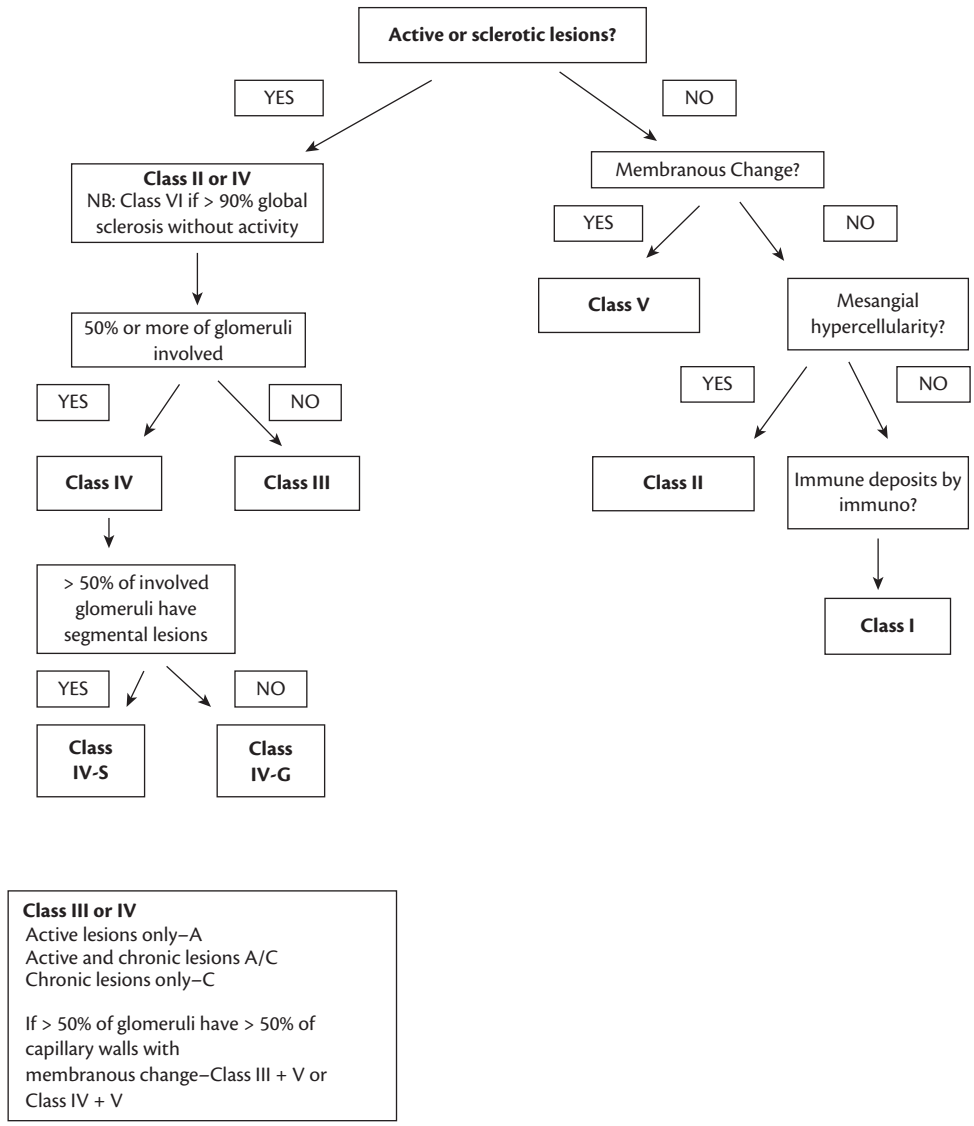

Fig. 162.6 Flowchart approach of the ISN/RPS classification.

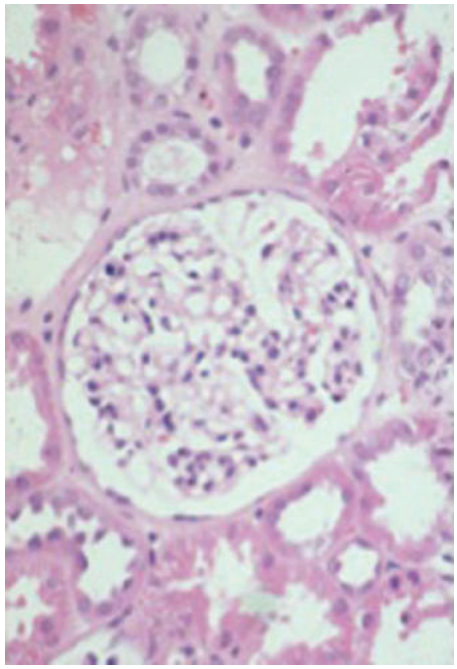

Fig. 162.7 Periodic acid–Schiff stain of class I LN on LM (no definable abnormality).

proliferative LN. Changes exist in varying degrees when examined by LM. IF identifies the so-called full house of deposits. EM identifies granular deposits either exclusively or predominantly in the mesangial area, as seen in Fig. 162.10. Occasional scattered small deposits within the subendothelial space may also be present on IF or EM examination. Specific therapy is usually not indicated unless there is transformation to higher classes of disease, hence regular surveillance for early identification of worsening proteinuria and/or renal impairment is therefore mandatory with a low threshold for repeat renal biopsy.

Class III and IV lupus nephritis

In these categories there are either 'active' or 'sclerotic' lesions visible on LM, which involve the capillary loops or Bowman's space and are associated with subendothelial immune deposits (Fig. 162.11).

Active lesions include endocapillary hypercellularity (Fig. 162.12), with or without leucocyte infiltration, and with substantial luminal reduction (Fig. 162.12); karyorrhexis (Figs 162.13 and 162.14); fibrinoid necrosis (Fig. 162.15); rupture of GBM; crescents (cellular (Fig. 162.16) or fibrocellular); subendothelial deposits identifiable by LM (wire loop); and intraluminal immune aggregates (hyaline thrombi).

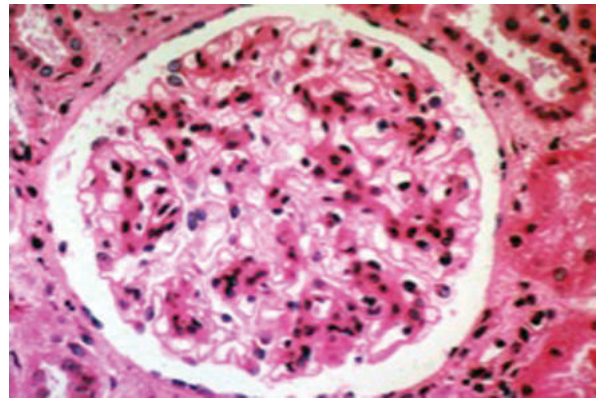

Fig. 162.9 Periodic acid–Schiff stain of class II LN demonstrating mesangial hypercellularity.

Glomerular crescents are a frequent finding in class III and IV LN and are a marker of severe glomerular injury. Cellular and fibrocellular crescents are indicative of reversibility whereas fibrous crescents (Fig. 162.19) suggest chronicity and irreversibility. When > 50% of the glomeruli exhibit crescentic change the pathological diagnosis is that of crescentic glomerulonephritis. On rare occasions a rapidly progressive crescentic glomerulonephritis may be observed with corresponding progressive renal impairment. Very occasional reports of a necrotizing pauci-immune glomerulonephritis have been documented in patients with lupus. Serological markers of SLE activity are normal or marginally abnormal and antineutrophil cytoplasmic antibodies are undetectable with absence of immune complex deposition on renal biopsy (Charney et al., 2000). The mesangial deposition of IgA and C3 has also been identified in lupus biopsies and as for many cases of idiopathic IgA disease, an indolent and often benign disease course has been demonstrated.

Sclerotic lesions include glomerular sclerosis (Fig. 162.18) (segmental, global), fibrous adhesions, and fibrous crescents.

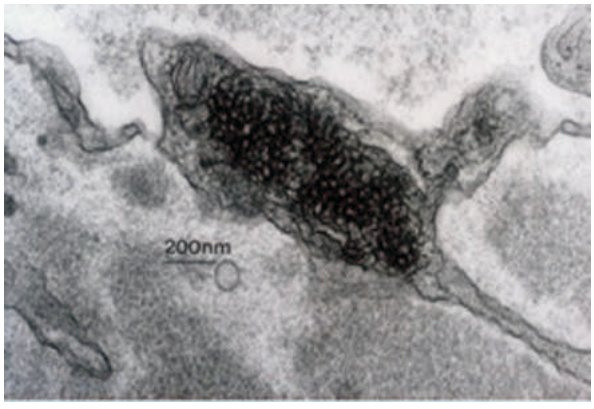

Fig. 162.8 EM demonstrating tubuloreticular inclusion.

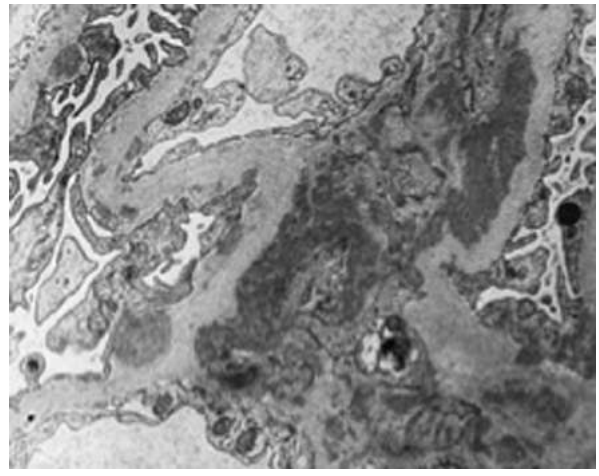

Fig. 162.10 EM of class II LN demonstrating mesangial electron dense deposits.

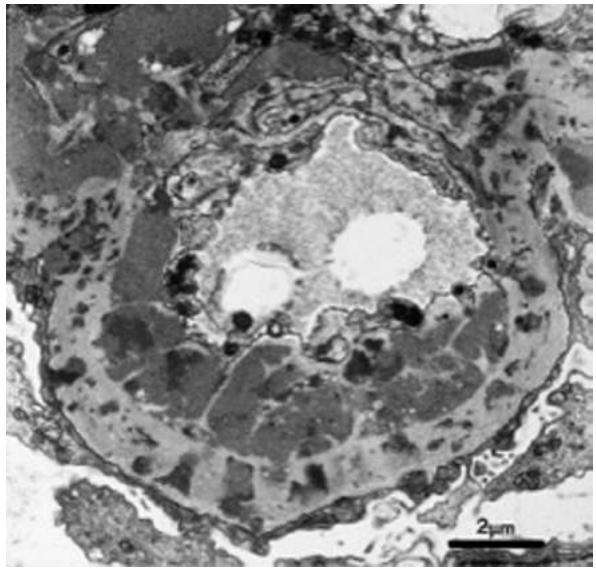

Fig. 162.11 EM demonstrating electron dense deposits in the mesangium, subendothelial, and subepithelial: highly suggestive of LN.

Class III lesions are focal affecting < 50% of all glomeruli. All immunoglobulin and complement components present in class II lesions are present in class III lesions, as are gamma and kappa light chains. Complexes are deposited in the mesangium of all the glomeruli irrespective of the pattern of proliferation. Coexistent TMA, suggested by fibrinogen deposits within capillaries, or tubulointerstitial disease may occur. Prognosis is variable with some cases remaining unchanged, some progressing to class IV or V lesions and others recovering. Class III is subdivided according to presence/absence of active and sclerotic lesions as follows: III (A) purely active lesions, III (A/C) active and chronic lesions, and III (C) chronic inactive with glomerular scars.

Class IV lesions are diffuse, affecting > 50% of the glomeruli and are subdivided, depending on their segmental or global nature, into class IV-S (Fig. 162.20) and IV-G respectively. It is the most common and severe form of LN affecting 20–60% of patients. Any and all of the active features of LN may be present to varying degrees in class IV-G disease. In class IV-S lesions, segmental endocapillary proliferation is observed with or without necrosis. IV-S or IV-G subdivision was incorporated into the ISN/RPS classification system following a long-term outcome study, which revealed evidence that class IV-S lesions exhibit a worse prognosis than class IV-G or combined class V + IV-G (Najafi et al., 2001). This disputes the long-held opinion that histopathological and clinical manifestations exist on a continuum and raised the question of an alternative pathogenic mechanism (Hill et al., 2005). Subsequent evidence regarding the outcomes of class IV-S and IV-G lesions has been conflicting. Although clinical and pathological variability has been documented, several studies have failed to identify a significant difference in outcome between classes (Mittal et al., 2004; Markowitz and D'Agati, 2007), some have reproduced evidence of poorer outcome in class IV-S disease (Yokoyama et al., 2004) whilst others report worse outcomes in class IV-G disease (Hill et al., 2005). Perhaps the most compelling data come from Mel Schwartz's group who have argued that it is the 'larger' segmental lesions, which predict the worst outcome (Schwartz et al., 2008) and that although these will be classified as 'Global' in the ISN classification they are different qualitatively from a true global lesion. The large segmental lesion tends to be relatively pauci-immune and necrotic compared to the highly proliferative with large deposit lesions seen in true global lesions (Schwartz et al., 2008). As with class III, class IV is subdivided according to proportion of active and sclerotic lesions as follows: IV (A) purely active lesions, IV (A/C) active and chronic lesions, and IV (C) chronic inactive with glomerular scars.

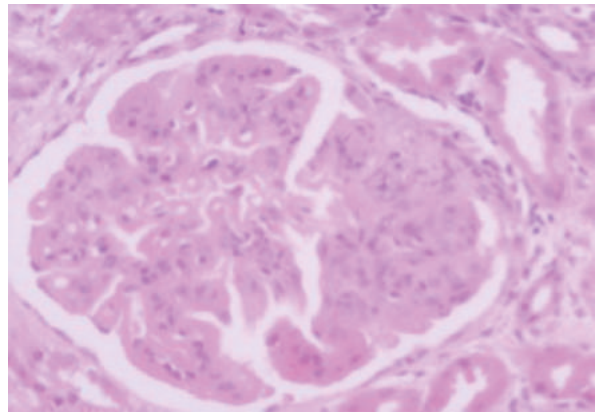

Fig. 162.13 Karyorrhexis: presence of apoptotic, pyknotic, and fragmented nuclei.

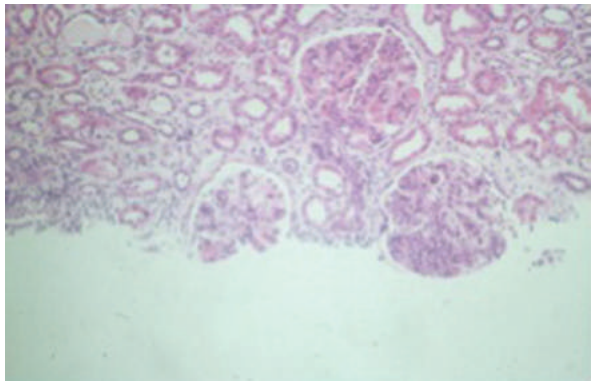

Fig. 162.12 LM of endocapillary hypercellularity with substantial luminal reduction.

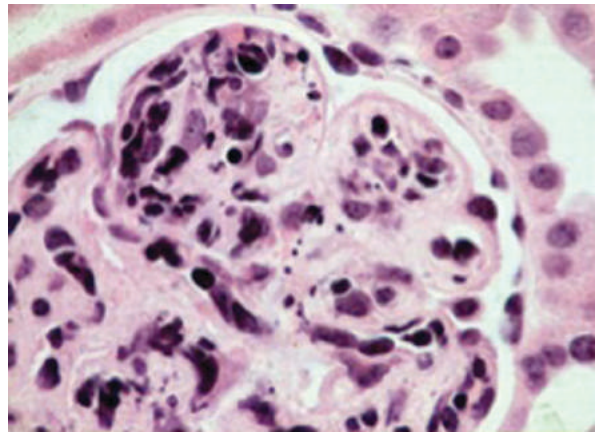

Fig. 162.14 Karyorrhexis: presence of apoptotic, pyknotic, and fragmented nuclei.

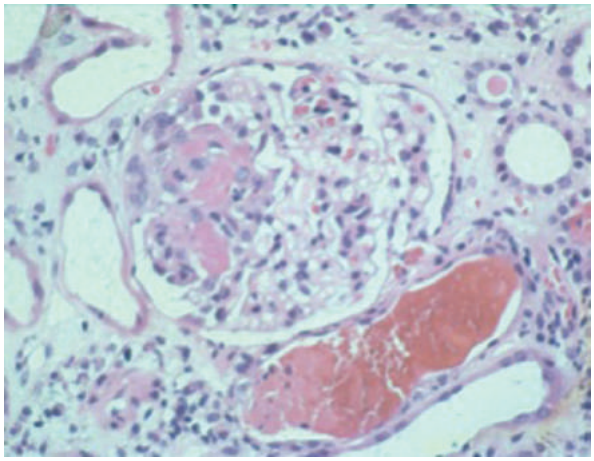

Fig. 162.15: LM of fibrinoid necrosis.

Class V lupus nephritis

Class V lesions are reported to have an incidence of between 10% and 30% and occur due to granular subepithelial immune complex deposition (Fig. 162.22) in either a segmental or global form affecting at least 50% of capillary basement membranes. This is accompanied by mesangial immune complex deposition and hypercellularity. The outcome is a membranous glomerulonephritis in which glomerular capillary walls appear diffusely thickened upon examination (Fig. 162.21). Early disease may only reveal abnormalities on IF. Advanced cases may show evidence of double contours within the capillary wall representative of intramembranous immune deposits and formation of new basement membranes. The so-called full house immune complex deposition is notable on IF within both the capillary walls and mesangium. The presence of scattered immune deposits within the subendothelial space may be demonstrated with IF or EM but if present on LM a diagnosis of combined class III plus V or IV plus V LN should be attributed, dependent on distribution. Chronicity causes glomerulosclerosis; however, where scarring results from previous proliferative changes the classification would be considered class III/IV(C) and V.

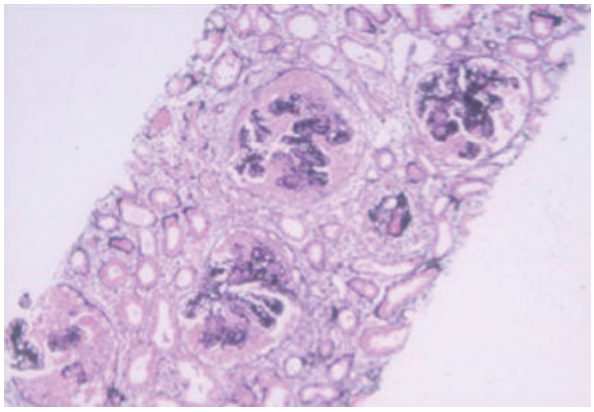

Fig. 162.16 Periodic acid–Schiff stain of cellular crescent under LM: defined by extracapillary cell proliferation of more than two cell layers occupying one-quarter or more of the glomerular capsular circumference.

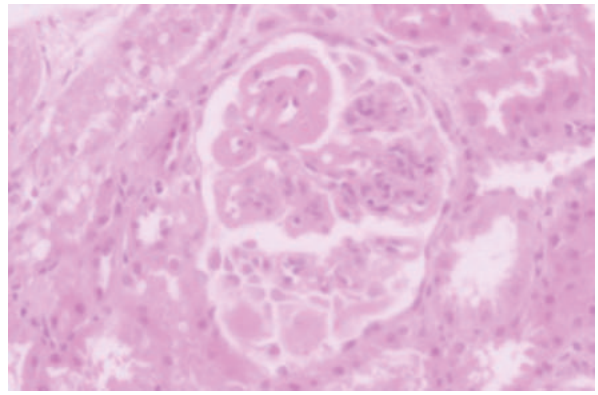

Fig. 162.17 'Wire loops'—subendothelial deposits visible by LM.

Class VI lupus nephritis

This represents advanced, often end-stage, LN occurring as a result of severe disease and accumulated injury due to numerous relapses of either class III, IV, or V LN. It is defined as global glomerulosclerosis affecting > 90% of glomeruli in the absence of any active lesions. Tubulointerstitial scarring and vascular sclerosis is notable on histological examination.

Transformation

Transformation from one class of nephritis to another is not an infrequent observation. It may represent a spontaneous occurrence or result from previous therapeutic intervention. Most commonly, lesions transform from lower to higher classes. Published results of repeat renal biopsy, in those with class II disease who are non-responders to treatment or who relapse following induction of remission, have reported very high rates of transformation (in certain populations) of class II lesions to higher-grade lesions (Tam et al., 2003). The consensus on transformation rates of class II lesions across all populations is estimated at 15–20%. It has been stipulated that this particular transformation merely represents progressive disease rather than a true transformation. Class III nephritis is documented to have the highest transformation rate with 20–40%

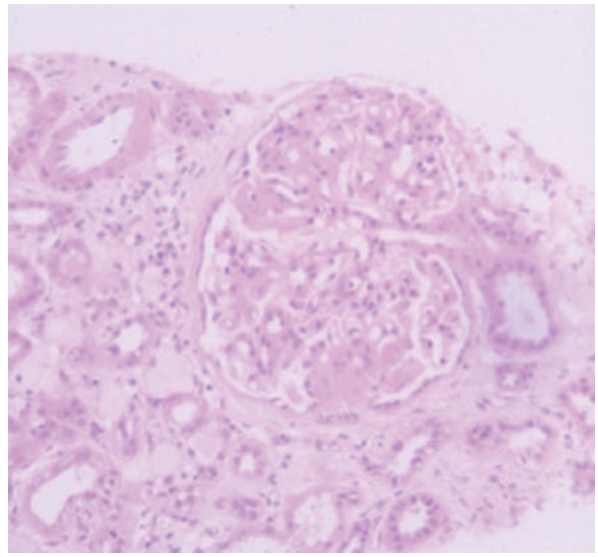

Fig. 162.18 Sclerotic lesions under LM: glomerular sclerosis.

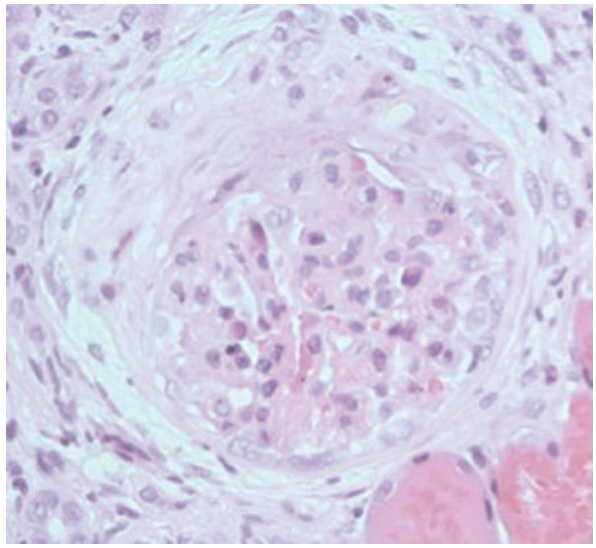

Fig. 162.19 Sclerotic lesions under LM: fibrous crescent.

developing features consistent with either class IV or V disease, although to the former most frequently. Class III lesions are therefore considered to be the most 'unstable' lesions. Respectively, the transformation of class V to class III or IV is reported to be an infrequent observation and has suggested possible variations in the underlying pathogenic mechanisms pertaining to membranous and proliferative disease. However, Mercadal et al. reported transformation from class V to IV in 35% of cases at 10 years (Mercadal et al., 2002).

Activity and chronicity—renal histology as a predictor of outcome

The activity and chronicity indices, indicative of the extent of reversible and irreversible lesions respectively, were developed by the US National Institutes of Health and based on the presence of typical pathological features associated with active and chronic forms of LN. They were introduced to guide therapy and aid in determining prognosis. Both indices employ a scoring system of 0–3 depending on the presence of appropriate features. Multiple investigating parties found the chronicity index particularly useful in predicting renal outcome in each case. However, it must be remembered that these indices related to treated disease—clearly, as Pollock's original data showed, untreated active (equivalent to class IV LN) predicts a very poor renal outcome. Activity indices on initial biopsy were found to be helpful in establishing therapeutic protocols rather than providing prognostic information. In 2000, Hill et al. established a new biopsy index using the glomerular activity, tubulointerstitial activity, chronic lesions, and immunofluorescence indices, which proved successful in better representing clinical and pathological correlations with outcome parameters (Hill et al., 2000). The same investigating group later undertook a large prospective study to examine the predictive value of these new indices. This study confirmed that whilst useful in establishing therapeutic protocols, pathological features present on initial biopsy were only modestly predictive of long-term outcome compared to features present on a second biopsy 6 months later, which proved to be strongly predictive of progressive renal impairment (Hill et al., 2001). The ISN/RSP classification of LN strongly emphasizes detailed microscopic description and qualitative and quantitative assessment of all active and chronic glomerular lesions as well as tubulointerstitial and vascular involvement. However, it does not recommend a formal activity or chronicity index.

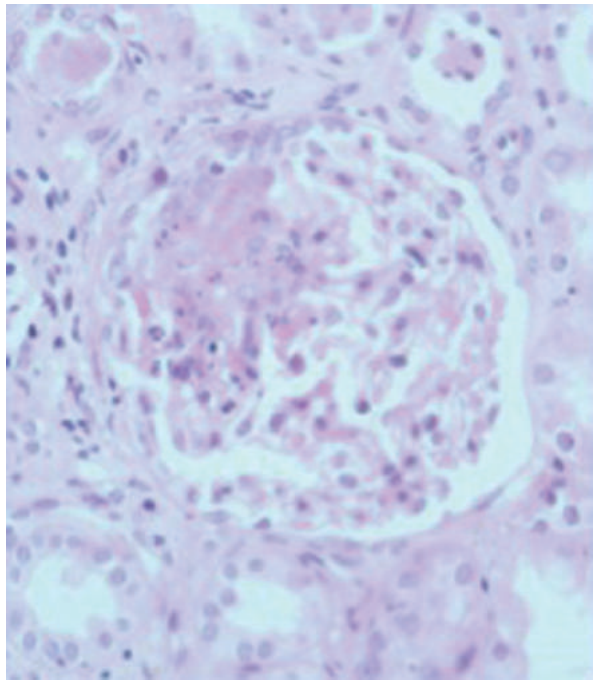

Fig. 162.20 Segmental glomerular lesion involving less than half of the glomerular tuft (i.e. at least half of the glomerular tuft is spared).

Renal lupus vasculopathies

A number of different types of vascular lesions affecting renal vessels of all sizes have been observed in LN and are adversely prognostic contributing to hypertension and progressive renal impairment. The ISN/RPS classification emphasizes the identification of the type, extent, and severity of lesions in context of their prognostic value.

Vascular immune complex deposition is most common, usually asymptomatic and uncomplicated. A disease-specific vasculopathy, characterized by non-inflammatory, eosinophilic intimal immune complex deposition within arterioles and inter-lobar arteries, can be identified. Destruction of the endothelial layer occurs with massive precipitation of all three immunoglobulins but predominantly IgG with C3. In severe forms, fibrin deposition ruptures the elastic membrane allowing spread of deposits into the media causing fibrinoid necrosis with luminal occlusion.

TMA has a reported incidence of 1–8% in patients with LN. It may occur in acute or chronic forms affecting the glomeruli and/or arterial vessels and may develop independently of disease activity or even in the absence of other parenchymal disease. Sudden onset of accelerated hypertension may represent onset of TMA. Renal prognosis is poor. Intraglomerular thrombotic lesions associated with endothelial damage indicate the presence of aPL. Renal vein thrombosis may also occur in the context of aPL or in those with nephrotic range proteinuria. True inflammatory vascular lesions are rare, occurring in < 5% of cases.

Other lesions of lupus nephritis

Tubulointerstitial disease is found in 60–70% of renal biopsies and may or may not be accompanied by immune complex deposition within tubular basement membranes and vasculature, detectable

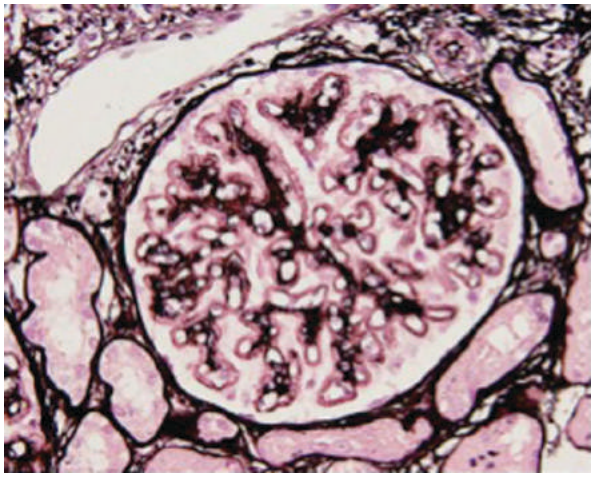

Fig. 162.21 Class V LN—silver stain demonstrating thickened GBM. Subepithelial deposits evident on EM.

on EM or IF. It most commonly occurs in association with active proliferative lesions of class III or IV disease. The severity of tubulointerstitial inflammation is a very important prognostic sign, correlating strongly with renal impairment, hypertension, and progressive renal disease. Rarely, isolated tubulointerstitial disease has been observed, manifesting clinically as distal renal tubular acidosis, hyperkalaemia due to impaired tubular secretion of potassium, or hypokalaemia in the presence of a salt-losing nephropathy. In very severe cases acute kidney injury may manifest. The ISN/RSP classification of LN emphasizes the importance of noting the presence, extent, and severity of tubulointerstitial disease in each case.

Glomerular podocytopathy with evidence of epithelial cell foot process effacement in the absence of immune complex deposition has also been reported, features characteristic of minimal change disease. Focal segmental glomerulosclerosis has also been reported. These changes may reflect cytokine mediated podocyte injury. The theory that such lesions exist as a separate and coincidental entity was dispelled by a study which retrospectively examined 470 renal biopsies of patients with SLE and demonstrated a significantly higher prevalence of podocytopathy than the projected 1 in 10,000 (Kraft et al., 2005). Other lesions reported include IgM nephropathy, thin membrane disease, and hypertensive nephropathy.

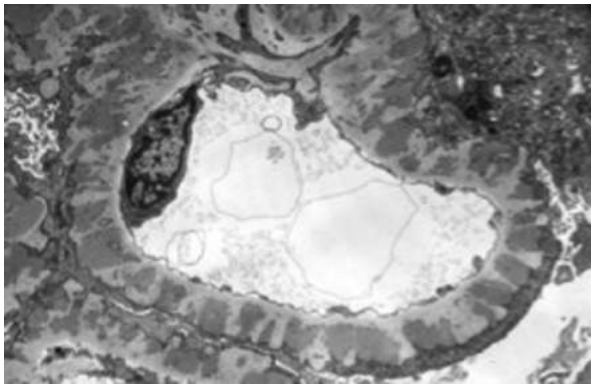

Fig. 162.22 Class V LN—silver stain demonstrating thickened GBM. Subepithelial deposits evident on EM.

Table 162.1 Clinical and immunologic criteria used in the SLICC classification system

Clinical criteria
1. Acute or subacute cutaneous lupus
2. Chronic cutaneous lupus
3. Oral/nasal ulcers
4. Non-scarring alopecia
5. Inflammatory synovitis with physician-observed swelling of two or more joints *or* tender joints with morning stiffness
6. Serositis
7. Renal: urine protein/creatinine (or 24-hour urine protein) representing at least 500 mg of protein/24 hours or red blood cell casts
8. Neurologic: seizures, psychosis, mononeuritis multiplex, myelitis, peripheral or cranial neuropathy, cerebritis (acute confusional state)
9. Haemolytic anaemia
10. Leucopenia (< 4000/mm^3 at least once) *or* lymphopenia (<1000/mm^3 at least once)
11. Thrombocytopenia (<100,000/mm^3) at least once
Immunologic criteria
1. ANA above laboratory reference range
2. Anti-dsDNA above laboratory reference range (except ELISA: twice above laboratory reference range)
3. Anti-Sm
4. Antiphospholipid antibody:
lupus anticoagulant
false-positive test for syphilis
anticardiolipin–at least twice normal or medium-high titre
anti-b2 glycoprotein 1
5. Low complement—low C3, low C4, low CH50
6. Direct Coombs test in absence of haemolytic anaemia

Classify a patient as having SLE if: the patient has biopsy-proven lupus nephritis with ANA or anti-dsDNA *or* the patient satisfies four of the criteria, including at least one clinical and one immunologic criterion.

Reproduced from Petri, M., Orbai, A. M., Alarcon, G. S., *et al*. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus, 64, 2012, 2677–86. Copyright 2012 *Arthritis and Rheumatism*. This material is reproduced with permission of John Wiley & Sons, Inc.

Diagnosis

The diagnosis of SLE is based upon the presence of clinical and/or laboratory features and immunological markers. The diagnostic criteria formulated by American Rheumatology Association (now known as the American College of Rhematologists (ACR)) in 1971 were modified in 1982 and again in 1997. It stipulated that a diagnosis of SLE be attributed if four or more of the listed diagnostic criteria exist either simultaneously or consecutively in one individual. New and improving knowledge of autoantibodies and the importance of neurological manifestations and complement consumption led to further revision and inclusion of the aforementioned in the 2012 Systemic Lupus International Collaborating Clinic

(SLICC) criteria (Table 162.1) (Petri et al., 2012). Using these criteria patients are diagnosed with SLE if the patient has biopsy-proven LN with ANA or anti-dsDNA or the patient satisfies four of the criteria, including at least one clinical and one immunologic criterion. However, in 2012, the ACR updated its guidelines on the screening, treatment, and management of LN, so that a 'renal biopsy sample demonstrating immune complex-mediated glomerulonephritis compatible with LN' is now an independent diagnostic criterion (Hahn et al., 2012).

Definitions of renal remission and relapse

There have been various trials in LN published over recent years with varying descriptions of remission and relapse (Wofsy et al., 2012). As per KDIGO 2012 guidance (KDIGO GN Work Group, 2012) the definition of complete response is: return of serum creatinine (SCr) to previous baseline, plus a decline in the urinary protein:creatinine ratio (UPCR) to < 500 mg/g (< 50 mg/mmol). Partial response: stabilization (± 25%), or improvement of SCr, but not to normal, plus a > 50% decrease in UPCR. If there was nephrotic-range proteinuria (UPCR > 3000 mg/g (> 300 mg/mmol)), improvement requires a > 50% reduction in UPCR, and a UPCR <3000 mg/g (< 300 mg/mmol). Deterioration: there is no definition of deterioration in LN to define treatment failure that has been tested prospectively as an indication to change in initial therapy. A sustained 25% increase in SCr is widely used but has not been validated (Radhakrishnan and Cattran, 2012).

A mild renal relapse may only be indicated by recurrence of microscopic haematuria, whereas as moderate to severe relapse will be indicated by an increase in proteinuria and/or a rise in SCr. The percentage change from baseline proteinuria/creatinine will indicate the severity of the relapse (Clough et al., 1990; Linnik et al., 2005; Rovin et al., 2005). Consider a repeat kidney biopsy during relapse if there is suspicion that the histologic class of LN has changed, or there is uncertainty whether a rising SCr and/or worsening proteinuria represents disease activity or chronicity.

References

Adler, M., Chambers, S., Edwards, C., *et al.* (2006). An assessment of renal failure in an SLE cohort with special reference to ethnicity, over a 25-year period. *Rheumatology*, 45, 1144–7.

Adu, D., Williams, D. G., Taube, D., *et al.* (1983). Late onset systemic lupus erythematosus and lupus-like disease in patients with apparent idiopathic glomerulonephritis. *QJM*, 52, 471–87.

Appel, G. B., Silva, F. G., Pirani, C. L., *et al.* (1978). Renal involvement in systemic lupus erythematosus (SLE): a study of 56 patients emphasizing histologic classification. *Medicine*, 57, 371–410.

Bencivelli, W., Vitali, C., Isenberg, D. A., *et al.* (1992). Disease activity in systemic lupus erythematosus: report of the Consensus Study Group of the European Workshop for Rheumatology Research. III. Development of a computerised clinical chart and its application to the comparison of different indices of disease activity. The European Consensus Study Group for Disease Activity in SLE. *Clin Exp Rheumat*, 10, 549–54.

Bertsias, G. K., Ioannidis, J. P., Aringer, M., *et al.* (2010). EULAR recommendations for the management of systemic lupus erythematosus with neuropsychiatric manifestations: report of a task force of the EULAR standing committee for clinical affairs. *Ann Rheumat Dis*, 69, 2074–82.

Bertsias, G. K., Tektonidou, M., Amoura, Z., *et al.* (2012). Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheumat Dis*, 71, 1771–82.

Cameron, J. S. (1999). Lupus nephritis. *J Am Soc Nephrol*, 10, 413–24.

Castrucci, G., Alimandi, L., Fichera, A., *et al.* (1990). (Changes in esophageal motility in patients with systemic lupus erythematosus: an esophago-manometric study). *Minerva Dietol Gastroenterol*, 36, 3–7.

Cervera, R., Khamashta, M. A., Font, J., *et al.* (1993). Systemic lupus erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European Working Party on Systemic Lupus Erythematosus. *Medicine*, 72, 113–24.

Cervera, R., Khamashta, M. A., Font, J., *et al.* (2003). Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. *Medicine*, 82, 299–308.

Chan, M. T., Owen, P., Dunphy, J., *et al.* (2008). Associations of erosive arthritis with anti-cyclic citrullinated peptide antibodies and MHC Class II alleles in systemic lupus erythematosus. *J Rheumatol*, 35, 77–83.

Charney, D. A., Nassar, G., Truong, L., *et al.* (2000). 'Pauci-Immune' proliferative and necrotizing glomerulonephritis with thrombotic microangiopathy in patients with systemic lupus erythematosus and lupus-like syndrome. *Am J Kidney Dis*, 35, 1193–206.

Chowdhary, V. R., Crowson, C. S., Poterucha, J. J., *et al.* (2008). Liver involvement in systemic lupus erythematosus: case review of 40 patients. *J Rheumatol*, 35, 2159–64.

Christopher-Stine, L., Siedner, M., Lin, J., *et al.* (2007). Renal biopsy in lupus patients with low levels of proteinuria. *J Rheumatol*, 34, 332–5.

Clough, J. D., Lewis, E. J., and Lachin, J. M. (1990). Treatment protocols of the lupus nephritis collaborative study of plasmapheresis in severe lupus nephritis. The Lupus Nephritis Collaborative Study Group. *Prog Clin Biol Res*, 337, 301–7.

Crestani, B. (2005). The respiratory system in connective tissue disorders. *Allergy*, 60, 715–34.

Doria, A., Iaccarino, L., Sarzi-Puttini, P., *et al.* (2005). Cardiac involvement in systemic lupus erythematosus. *Lupus*, 14, 683–6.

Ebert, E. C. and Hagspiel, K. D. (2011). Gastrointestinal and hepatic manifestations of systemic lupus erythematosus. *J Clin Gastroenterol*, 45, 436–41.

Elliot, V., Cairns, T., and Cook, H. T. (2006). Evolution of lesions over 10 years in a patient with SLE: flowchart approach to the new International Society of Nephrology (ISN)/Renal Pathology Society (RPS) classification of lupus nephritis. *Am J Kidney Dis*, 47, 184–90.

Fessler, B. J. and Boumpas, D. T. (1995). Severe major organ involvement in systemic lupus erythematosus. Diagnosis and management. *Rheumatic Dis Clin North Am*, 21, 81–98.

Franceschini, F., Cretti, L., Quinzanini, M., *et al.* (1994). Deforming arthropathy of the hands in systemic lupus erythematosus is associated with antibodies to SSA/Ro and to SSB/La. *Lupus*, 3, 419–22.

Fries, J. W., Mendrick, D. L., and Rennke, H. G. (1988). Determinants of immune complex-mediated glomerulonephritis. *Kidney Int*, 34, 333–45.

Futrell, N., Schultz, L. R., and Millikan, C. (1992). Central nervous system disease in patients with systemic lupus erythematosus. *Neurology*, 42, 1649–57.

Griffin, M. R. and Smalley, W. E. (1995). Drugs and ulcers: clues about mucosal protection from epidemiologic studies. *J Clin Gastroenterol*, 21 Suppl 1, S113–19.

Grossman, J. M. (2009). Lupus arthritis. *Best Pract Res Clin Rheumatol*, 23, 495–506.

Hahn, B. H., McMahon, M. A., Wilkinson, A., *et al.* (2012). American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res*, 64, 797–808.

Hill, G. S., Delahousse, M., Nochy, D., *et al.* (2000). A new morphologic index for the evaluation of renal biopsies in lupus nephritis. *Kidney Int*, 58, 1160–73.

Hill, G. S., Delahousse, M., Nochy, D., *et al.* (2001). Predictive power of the second renal biopsy in lupus nephritis: significance of macrophages. *Kidney Int*, 59, 304–16.

Hill, G. S., Delahousse, M., Nochy, D., *et al.* (2005). Class IV-S versus class IV-G lupus nephritis: clinical and morphologic differences suggesting different pathogenesis. *Kidney Int*, 68, 2288–97.

Joseph, F. G., Lammie, G. A., and Scolding, N. J. (2007). CNS lupus: a study of 41 patients. *Neurology*, 69, 644–54.
Kasitanon, N., Magder, L. S., and Petri, M. (2006). Predictors of survival in systemic lupus erythematosus. *Medicine*, 85, 147–56.
KDIGO GN Work Group. (2012). KDIGO clinical practice guideline for glomerulonephritis. *Kidney Int Supplement*, 2, 139–274.
Keeling, D. M. and Isenberg, D. A. (1993). Haematological manifestations of systemic lupus erythematosus. *Blood Rev*, 7, 199–207.
Kraft, S. W., Schwartz, M. M., Korbet, S. M., *et al.* (2005). Glomerular podocytopathy in patients with systemic lupus erythematosus. *J Am Soc Nephrol*, 16, 175–9.
Linnik, M. D., Hu, J. Z., Heilbrunn, K. R., *et al.* (2005). Relationship between anti-double-stranded DNA antibodies and exacerbation of renal disease in patients with systemic lupus erythematosus. *Arthritis Rheum*, 52, 1129–37.
Markowitz, G. S. and D'Agati, V. D. (2007). The ISN/RPS 2003 classification of lupus nephritis: an assessment at 3 years. *Kidney Int*, 71, 491–5.
Martinez-Banos, D., Crispin, J. C., Lazo-Langner, A., *et al.* (2006). Moderate and severe neutropenia in patients with systemic lupus erythematosus. *Rheumatology*, 45, 994–8.
Matsumoto, T., Yoshimine, T., Shimouchi, K., *et al.* (1992). The liver in systemic lupus erythematosus: pathologic analysis of 52 cases and review of Japanese Autopsy Registry Data. *Hum Pathol*, 23, 1151–8.
Mercadal, L., Montcel, S. T., Nochy, D., *et al.* (2002). Factors affecting outcome and prognosis in membranous lupus nephropathy. *Nephrol Dial Transplant*, 17, 1771–8.
Mikdashi, J., Handwerger, B., Langenberg, P., *et al.* (2007). Baseline disease activity, hyperlipidemia, and hypertension are predictive factors for ischemic stroke and stroke severity in systemic lupus erythematosus. *Stroke*, 38, 281–5.
Mittal, B., Hurwitz, S., Rennke, H., *et al.* (2004). New subcategories of class IV lupus nephritis: are there clinical, histologic, and outcome differences? *Am J Kidney Dis*, 44, 1050–9.
Moder, K. G., Miller, T. D., and Tazelaar, H. D. (1999). Cardiac involvement in systemic lupus erythematosus. *Mayo Clinic Proc*, 74, 275–84.
Najafi, C. C., Korbet, S. M., Lewis, E. J., *et al.* (2001). Significance of histologic patterns of glomerular injury upon long-term prognosis in severe lupus glomerulonephritis. *Kidney Int*, 59, 2156–63.
Nossent, H. C., Henzen-Logmans, S. C., Vroom, T. M., *et al.* (1990). Contribution of renal biopsy data in predicting outcome in lupus nephritis. Analysis of 116 patients. *Arthritis Rheum*, 33, 970–7.
Nossent, J. C. and Swaak, A. J. (1991). Prevalence and significance of haematological abnormalities in patients with systemic lupus erythematosus. *QJM*, 80, 605–12.
Ostendorf, B., Scherer, A., Specker, C., *et al.* (2003). Jaccoud's arthropathy in systemic lupus erythematosus: differentiation of deforming and erosive patterns by magnetic resonance imaging. *Arthritis Rheum*, 48, 157–65.
Patel, M., Clarke, A. M., Bruce, I. N., *et al.* (2006). The prevalence and incidence of biopsy-proven lupus nephritis in the UK: evidence of an ethnic gradient. *Arthritis Rheum*, 54, 2963–9.
Petri, M., Orbai, A. M., Alarcon, G. S., *et al.* (2012). Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum*, 64, 2677–86.
Pistiner, M., Wallace, D. J., Nessim, S., *et al.* (1991). Lupus erythematosus in the 1980s: a survey of 570 patients. *Semin Arthritis Rheum*, 21, 55–64.
Pollak, V. E., Pirani, C. L., and Schwartz, F. D. (1964). The natural history of the renal manifestations of systemic lupus erythematosus. *J Lab Clin Med*, 63, 537–50.
Radhakrishnan, J. and Cattran, D. C. (2012). The KDIGO practice guideline on glomerulonephritis: reading between the (guide)lines—application to the individual patient. *Kidney Int*, 82, 840–56.
Rahman, P., Gladman, D. D., and Urowitz, M. B. (2000). Premature coronary artery disease in systemic lupus erythematosus in the absence of corticosteroid use. *J Rheumatol*, 27, 1323–5.
Ravelli, A., Di Fuccia, G., Caporali, R., *et al.* (1993). Severe retinopathy in systemic lupus erythematosus associated with IgG anticardiolipin antibodies. *Acta Paediatr*, 82, 624–6.
Roldan, C. A., Qualls, C. R., Sopko, K. S., *et al.* (2008). Transthoracic versus transesophageal echocardiography for detection of Libman-Sacks endocarditis: a randomized controlled study. *J Rheumatol*, 35, 224–9.
Roldan, C. A., Shively, B. K., and Crawford, M. H. (1996). An echocardiographic study of valvular heart disease associated with systemic lupus erythematosus. *N Engl J Med*, 335, 1424–30.
Rothfield, N., Sontheimer, R. D., and Bernstein, M. (2006). Lupus erythematosus: systemic and cutaneous manifestations. *Clin Dermatol*, 24, 348–62.
Rovin, B. H., Song, H., Birmingham, D. J., *et al.* (2005). Urine chemokines as biomarkers of human systemic lupus erythematosus activity. *J Am Soc Nephrol*, 16, 467–73.
Schneebaum, A. B., Singleton, J. D., West, S. G., *et al.* (1991). Association of psychiatric manifestations with antibodies to ribosomal P proteins in systemic lupus erythematosus. *J Am Med*, 90, 54–62.
Schwartz, M. M., Korbet, S. M., and Lewis, E. J. (2008). The prognosis and pathogenesis of severe lupus glomerulonephritis. *Nephrol Dial Transplant*, 23, 1298–306.
Seligman, V. A., Lum, R. F., Olson, J. L., *et al.* (2002). Demographic differences in the development of lupus nephritis: a retrospective analysis. *J Am Med*, 112, 726–9.
Seshan, S. V. and Jennette, J. C. (2009). Renal disease in systemic lupus erythematosus with emphasis on classification of lupus glomerulonephritis: advances and implications. *Arch Pathol Lab Med*, 133, 233–48.
Sibley, J. T., Olszynski, W. P., Decoteau, W. E., *et al.* (1992). The incidence and prognosis of central nervous system disease in systemic lupus erythematosus. *J Rheumatol*, 19, 47–52.
Sivaraj, R. R., Durrani, O. M., Denniston, A. K., *et al.* (2007). Ocular manifestations of systemic lupus erythematosus. *Rheumatology*, 46, 1757–62.
Sontheimer, R. D. (1997). The lexicon of cutaneous lupus erythematosus—a review and personal perspective on the nomenclature and classification of the cutaneous manifestations of lupus erythematosus. *Lupus*, 6, 84–95.
Streck Ade, S., Staub, H. L., De Freitas, C. Z., *et al.* (2012). Posterior reversible encephalopathy syndrome (PRES) and systemic lupus erythematosus: report of two cases. *Rev Bras Reumatol*, 52, 804–10.
Sultan, S. M., Ioannou, Y., and Isenberg, D. A. (1999). A review of gastrointestinal manifestations of systemic lupus erythematosus. *Rheumatology*, 38, 917–32.
Tam, L. S., Li, E. K., Lai, F. M., *et al.* (2003). Mesangial lupus nephritis in Chinese is associated with a high rate of transformation to higher grade nephritis. *Lupus*, 12, 665–71.
Torre, O. and Harari, S. (2011). Pleural and pulmonary involvement in systemic lupus erythematosus. *Presse Med*, 40, e19–29.
Toubi, E., Khamashta, M. A., Panarra, A., *et al.* (1995). Association of antiphospholipid antibodies with central nervous system disease in systemic lupus erythematosus. *J Am Med*, 99, 397–401.
Van Vugt, R. M., Derksen, R. H., Kater, L., *et al.* (1998). Deforming arthropathy or lupus and rhupus hands in systemic lupus erythematosus. *Ann Rheumat Dis*, 57, 540–4.
Vina, E. R., Fang, A. J., Wallace, D. J., *et al.* (2005). Chronic inflammatory demyelinating polyneuropathy in patients with systemic lupus erythematosus: prognosis and outcome. *Semin Arthritis Rheum*, 35, 175–84.
Von Feldt, J. M. (1995). Systemic lupus erythematosus. Recognizing its various presentations. *Postgrad Med*, 97, 79, 83, 86 passim.
Vozmediano, C., Rivera, F., Lopez-Gomez, J. M., *et al.* (2012). Risk factors for renal failure in patients with lupus nephritis: data from the Spanish registry of glomerulonephritis. *Nephron Extra*, 2, 269–77.
Wahl, D. G., Guillemin, F., De Maistre, E., *et al.* (1998). Meta-analysis of the risk of venous thrombosis in individuals with antiphospholipid antibodies without underlying autoimmune disease or previous thrombosis. *Lupus*, 7, 15–22.
Wang, B., Gladman, D. D., and Urowitz, M. B. (1998). Fatigue in lupus is not correlated with disease activity. *J Rheumatol*, 25, 892–5.

Ward, M. M. and Studenski, S. (1991). The time course of acute psychiatric episodes in systemic lupus erythematosus. *J Rheumatol*, 18, 535–9.

Weening, J. J., D'Agati, V. D., Schwartz, M. M., *et al.* (2004). The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol*, 15, 241–50.

Wofsy, D., Hillson, J. L., and Diamond, B. (2012). Abatacept for lupus nephritis: alternative definitions of complete response support conflicting conclusions. *Arthritis Rheum*, 64, 3660–5.

Wolfe, F., Petri, M., Alarcon, G. S., *et al.* (2009). Fibromyalgia, systemic lupus erythematosus (SLE), and evaluation of SLE activity. *J Rheumatol*, 36, 82–8.

Wong, K. L., Woo, E. K., Yu, Y. L., *et al.* (1991). Neurological manifestations of systemic lupus erythematosus: a prospective study. *QJM*, 81, 857–70.

Yin, Y., Wu, X., Shan, G., *et al.* (2012). Diagnostic value of serum anti-C1q antibodies in patients with lupus nephritis: a meta-analysis. *Lupus*, 21, 1088–97.

Yokoyama, H., Wada, T., Hara, A., *et al.* (2004). The outcome and a new ISN/RPS 2003 classification of lupus nephritis in Japanese. *Kidney Int*, 66, 2382–8.

CHAPTER 163

The patient with systemic lupus erythematosus: treatment and outcome

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Introduction

According to series and recruitment biases, between 30% and 60% of patients with systemic lupus erythematosus (SLE) suffer from renal involvement, either as an initial manifestation or later during the course of their disease (Cervera et al., 2003). Although patient survival has dramatically improved over the last 50 years, current treatment of lupus nephritis (LN) still achieves suboptimal results, with end-stage renal disease (ESRD) rates varying from 10% to 30% according to ethnicity, duration of follow-up, and series. Of note, the incidence of ESRD attributable to LN in the United States increased from 1.13 cases per million in 1982 to 4.9 cases per million in 2004, thereby clearly indicating an unmet need (Costenbader et al., 2011).

Disease heterogeneity makes treatment of lupus nephritis a real challenge

LN is highly heterogeneous. Initial clinical presentation ranges from asymptomatic proteinuria with or without haematuria, discovered by routine dipstick examination or urinalysis in a known SLE patient, to nephrotic syndrome with or without moderate or severe renal impairment, sometimes without many extrarenal signs. Thus, LN can be captured in early stages or diagnosed much later when the underlying immune process has already been at work for months. The variety of pathological changes on renal biopsy further contributes to heterogeneity. They encompass glomerular, tubulointerstitial, and (rarely) vascular changes of acute (mostly reversible) and/or chronic (mostly irreversible) nature. Lastly, the disease may run different courses: 'single shot', relapsing (35% of LN patients experience at least one episode of renal relapse), or refractory. All together, these figures explain that outcome is highly unpredictable, the more so as the need for renal replacement therapy (RRT) may occur decades after the first renal insult. Many poor prognostic factors have been identified, the most important being race (African Americans run the worst prognosis), poor socioeconomic status, chronic glomerular and interstitial changes on renal biopsy, renal relapses, uncontrolled hypertension, non-observance to therapy, and an absence of primary response (proteinuria drop) after 3–6 months of induction immunosuppression therapy (Houssiau et al., 2004).

Keeping heterogeneity in mind, treatment should ideally be patient-tailored, for example, based on biomarkers identified at baseline, which would accurately predict long-term renal prognosis (Rovin et al., 2009). While some (mainly urinary) biomarkers appear promising (e.g. monocyte chemoattractant protein-1 (MCP-1), neutrophil gelatinase-associated lipocalin (NGAL), or tumour necrosis factor (TNF)-like inducer of apoptosis (TWEAK)), none has been longitudinally validated in large cohorts of LN patients, across different ethnic backgrounds. Therefore, treatment decisions at the bedside remain not only evidence based but also eminence-where-needed based. Despite several well-conducted randomized clinical trials which have been performed within the last decade, thereby facilitating the decision-making process, a 'trial and error' approach remains relevant.

Treatment goals consist of (1) achieving a prompt renal response (proteinuria drop and recovery of a normal kidney function) by prescribing a stringent induction immunosuppressive regimen, for a period of 3–6 months, combining glucocorticoids (GCs) with another immunosuppressant (mostly cytotoxics such as cyclophosphamide (CY) or mycophenolate mofetil (MMF)); (2) maintaining this response by avoiding renal (and other organ) flares using long-term (3–10 years) immunosuppression, with low-dose GC and another immunosuppressant (mostly MMF or azathioprine (AZA)); (3) preventing renal impairment (*a fortiori* ESRD) including in the very long term; and (4) fulfilling these objectives with minimal toxicity, taking into account the patient's perspectives, such as pregnancy plans.

In this chapter, the pros and cons of classical and newer immunosuppressive regimens will be described, stressing the need for a more patient-friendly approach. Targeted therapies, based on concepts emerging from preclinical research, will be briefly reviewed. The need for optimal multidisciplinary care will be emphasized. Finally, selected issues regarding dealing with pregnancy and RRT in LN patients will be discussed.

Indications for immunosuppression

Not all LN patients require heavy immunosuppression. Thus, patients with International Society of Nephrology (ISN)/Renal Pathology Society (RPS) class I (glomerular immune deposits without hypercellularity) or class II (mild mesangial hypercellularity)

glomerulonephritis have minimal clinical disease (low-grade proteinuria) and run a low risk of poor renal outcome. Actually, class I/II LN are less likely to be diagnosed if the proteinuria cut-off for a renal biopsy is set at ≥ 0.5 g/day (as in most lupus centres). In those patients, clinical and laboratory follow-up is advised, since shifts towards more aggressive forms of LN can occur, in particular in patients with low complement and high anti-dsDNA antibody serum titres, who should be 'red flagged'. Class I/II LN patients should be given angiotensin-converting enzyme inhibitor (ACEI)/angiotensin receptor blocker (ARB) therapy for renal protection and hydroxychloroquine to control extrarenal signs (see later).

Patients with so-called proliferative nephritis, either focal (class III) or diffuse (class IV), either alone or with superimposed membranous nephritis (class V), require immunosuppression. The case of pure membranous disease is discussed separately.

Glucocorticoids

No drug ever has so dramatically improved survival of severe LN patients as GC. But few drugs are responsible for so many adverse events, sometimes improperly attributed to concomitantly prescribed cytotoxic drugs. As a logical consequence, patients' reluctance to take GC cannot be overlooked anymore. Yet, little attention has been given in clinical trials to the initial dose and tapering regimen of GC. For years, textbooks have advised starting with a standard prednisolone dose of 1 mg/kg/day. A first step forward was the suggestion, by the National Institutes of Health (NIH) investigators, to use intravenous (IV) pulse methylprednisolone (MP) therapy, mostly at a dose of 1 g (Gourley et al., 1996). This mode of administration achieves potent immunosuppression while minimizing at least some of the metabolic effects of oral GC. In recent trials, such as those performed by the Euro-Lupus group (Houssiau et al., 2002, 2010), a much lower dose of oral prednisolone (0.5 mg/kg/day) was used, in combination with a few IV MP pulses to launch therapy. Whether oral GC therapy can be completely skipped from induction immunosuppressive regimens is far from proven but it was recently suggested by an uncontrolled study which showed that complete renal remission can be achieved at 6 months in a significant percentage of cases without any oral GC therapy (patients were given pulse IV MP, rituximab, and MMF) (Condon et al., 2013). In any case, titration of the GC dose to the minimum now belongs to standard of care.

Cyclophosphamide

CY (in fact, its metabolites) is purported to exert its cytotoxic and immunosuppressive properties by alkylation of purine bases, thereby inducing DNA damage and interfering with cell division. Oral CY was successfully tested in LN by the Mayo Clinic investigators, in combination with GC (Donadio et al., 1972). However, the adverse effects of oral CY are so numerous and so potentially severe (cytopenia, infection, haemorrhagic cystitis, bladder and other cancers, premature gonadal failure) that this mode of administration is currently not recommended, except in a few highly selected severe LN cases, where it should be prescribed only for a short period of time (up to 3 months).

Somewhat ironically, because CY was specifically developed for oral administration, a clear shift has occurred towards the IV route, after the work by the NIH investigators who popularized the so-called NIH regimen consisting in monthly (for 6 months) followed by quarterly (for up to 1 year after complete remission) high-dose (0.75–1.5 g/m^2) IV CY pulses, mostly combined to IV MP pulses (Austin et al., 1986; Boumpas et al., 1992; Gourley et al., 1996). Of note, in the strict 'NIH regimen', the CY dose must be increased, pulse after pulse, until the total white blood cell count drops below 4000/μL on day 10–14. Patients require IV antiemetics, oral and IV hyperhydration, and, therefore, overnight hospital stay. Mesna® must be prescribed to prevent bladder toxicity (the drug binds to acrolein, one of the toxic CY metabolites). While this regimen was shown to be superior to GC to preserve renal function in the long run, the high incidence of severe infections and premature gonadal failure (between 35% and 50% of women at risk) made it very unpopular amongst young, female LN patients, in particular the quarterly maintenance protocol, which is hardly used anymore.

Alternatives to the 'NIH regimen' were generated, amongst which the so-called sequential Euro-Lupus regimen consisting in prescribing CY in low dose (fixed 500 mg) for a short period of time (3 months; six fortnightly pulses), before switching to a more patient-friendly drug, namely AZA (2 mg/kg/day) for chronic long-term (5–10 years) immunosuppression. The concept was successfully tested in a controlled trial, the Euro-Lupus Nephritis Trial (Houssiau et al., 2002). To summarize, after 10 years of follow-up, the Euro-Lupus regimen achieved results comparable to a NIH-like high-dose CY protocol, with a very low ESRD rate (5%) (Houssiau et al., 2010). Whether this conclusion, based on a European trial performed in a mainly Caucasian population, can be extrapolated to other ethnic and socioeconomic groups and to the most severe cases of LN is currently unknown. Conversely, nothing argues against this possibility and the many advantages of the Euro-Lupus regimen must be emphasized: no need for prolonged inpatient stay (500 mg IV CY can be administered as a 30-minute drip infusion in a day-clinic), for hyperhydration, for Mesna®, no white blood cells count (WBC) monitoring and no risk of premature gonadal failure.

On the whole, despite the drug not being labelled for LN, CY (in particular the more patient friendly IV and low-dose regimens), remains a valid option for immunosuppression in class III (± V) and class IV (± V) LN, keeping in mind that the IV route ensures optimal compliance and that very long-term follow-up is only available for IV CY protocols so far.

Mycophenolate mofetil

MMF, the prodrug of mycophenolic acid (MPA), has been used in transplantation medicine for at least 20 years to prevent graft rejection. MPA inhibits inosine monophosphate dehydrogenase, an enzyme involved in purine synthesis, but does not incorporate into DNA, and therefore should not induce mutagenic events. Side effects (gastrointestinal (GI) intolerance such as diarrhoea, haematological toxicity such as anaemia in patients with renal impairment) can usually be managed by dose adaptations and, on the whole, the toxicity profile of the drug is fairly good. Its teratogenicity forbids its use during pregnancy, at least in the first trimester (Anderka et al., 2009). Several doses have been tested in LN trials, mostly between 1.5 and 3g/day. MMF has been compared to oral and IV CY for induction therapy and to AZA for maintenance treatment.

MMF was found superior to NIH IV CY to induce complete remission of renal signs at 6 months (22.5% vs 5.8%) in a US

(> 50% African Americans) randomized trial (Ginzler et al., 2005). However, in a subsequent multiethnic trial (the ASPREVA Lupus Management Study (ALMS)), the two immunosuppressants were found to be equivalent in inducing a renal response at 6 months (56.2% in the MMF group vs 53.0% in the IV CY arm), with an advantage of MMF over IV CY in non-Caucasian/non-Asian patients (Appel et al., 2009). Interestingly, a subset analysis performed on patients presenting with a glomerular filtration rate (GFR) < 30 mL/min indicated that MMF was not less effective than IV CY in these more severely affected patients (Walsh et al., 2013). Side effects were comparable in both groups. To summarize, MMF can be considered as at least equivalent to CY in inducing an early renal response, although we lack long-term data on patients induced with MMF. The decision to start MMF or IV CY at the bedside will be influenced by many variables. Thus, if one anticipates non-observance to therapy, IV CY may be the ideal choice. Conversely, in patients with pregnancy plans and of non-Caucasian, non-Asian ethnicity, it may be wiser to prescribe MMF.

MMF was also proven effective for maintenance immunosuppression in LN (Contreras et al., 2004; Ginzler et al., 2010; Houssiau et al., 2010b). Thus, patients randomized in ALMS who had responded to MMF or IV CY at 6 months (± 50% of the original cohort), were re-randomized to receive AZA or MMF for an additional period of 3 years. Time to treatment failure (a composite endpoint) was statistically shorter for AZA patients compared to MMF patients and superiority of MMF over AZA was demonstrated for patients who had achieved a response at 6 months with MMF (36% vs 21% failure rate) or with IV CY (28% vs 11%). Side effects were comparable (Dooley et al., 2011). In the MAINTAIN Nephritis Trial performed in a Caucasian European population, all patients received the same induction regimen, namely GC and 6 fortnightly pulses of 500 mg IV CY (Euro-Lupus regimen). At month 3, irrespectively of the magnitude of their renal response, patients were switched to AZA or MMF. Time to renal flare did not differ at 5 years (19% and 25% flare rate for MMF and AZA patients, respectively). Untoward effects did not differ between the two groups except for transient cytopenias (more frequent in the AZA group) (Houssiau et al., 2010b). Repeat renal biopsies, performed 2 years after randomization, in a representative set of patients, failed to reveal an advantage of one drug over the other in terms of activity/chronicity indices (Stoenoiu et al., 2012). The design and the patient population of the two MMF/AZA maintenance LN trials are so different that one should avoid comparing them head-to-head. Rather, we would suggest interpreting the results in a positive way, since at least two drugs are available for long-term use in LN patients, with a reasonable efficacy and toxicity profile, although none of them are—again—licensed for use in LN.

Azathioprine

AZA is mostly used in systemic rheumatic diseases as a steroid-sparing drug. Its toxicity profile is rather safe but adverse events can lead to treatment discontinuation in case of severe GI intolerance, hepatitis, hypersensitivity reaction, or severe pancytopenia. The latter occurs mainly in patients who are deficient in thiopurine methyltransferase (TPMT), one of the enzymes responsible for the catabolism of AZA (actually of 6-MP) into inactive metabolites. When available, TPMT genotyping should be performed before AZA is prescribed: AZA should be started at lower doses in heterozygous patients and avoided in case of homozygous TPMT deficiency (Ford and Berg 2010). An increased risk of non-melanoma skin cancers is yet another concern linked to the very chronic use of AZA. In this respect, an increased frequency of somatic mutations at the hypoxanthine phosphoribosyltransferase locus, related to both total dose and treatment duration, was recently found in peripheral T lymphocytes of patients suffering from inflammatory bowel disease treated with AZA, thereby stressing the potential carcinogenicity of the drug (Nguyen et al., 2009). The place of the drug in the armamentarium of LN is mainly on the maintenance side where its competes with MMF (Houssiau et al., 2010b; Dooley et al., 2012). In this respect, it should be stressed that, contrary to MMF, AZA can be safely prescribed during pregnancy (Ramsay-Goldman and Schilling 1997), an issue that needs to be appreciated when long-term immunosuppression is considered in young women planning pregnancy. Whether AZA can be used, in conjunction with GC, as induction therapy of LN has been suggested but, at least compared to NIH IV CY, its use is associated with a higher renal relapse rate (Grootscholten et al., 2006), even if the percentage of patients with a sustained doubling of serum creatinine did not differ between groups after 10 years of follow-up.

Other immunosuppressants

Calcineurin inhibitors (ciclosporin microemulsion and tacrolimus) have logically been tested in LN based on their mode of action and the data gathered from renal transplantation (RT). Few randomized controlled trials, performed with small numbers of LN patients followed for a short period of time, suggest that both drugs can reduce proteinuria (Moroni et al., 2009; Lee et al., 2011). Some of their side effects, such as chronic nephrotoxicity, hypertension, diabetes, and impaired lipid profile (less marked with tacrolimus than with ciclosporin), obviously restrict their use in LN patients already at high cardiovascular risk, not to mention the fact that proteinuria mostly relapses when the drug is withdrawn. Keeping these caveats in mind, although calcineurin inhibitors can prove useful in selected cases of proliferative LN unresponsive to conventional therapy or of membranous LN, they are not considered as a first-choice therapy.

Based on the pathogenic role of autoantibodies and immune complexes in LN, repeated plasma exchanges have been proposed but were not found superior to a sham procedure, in addition to standard of care, in a randomized controlled trial (Lewis et al., 1992). Nevertheless, they can be useful as adjunct therapy in selected cases of refractory LN, in particular those associated with severe extrarenal manifestations or within the context of a catastrophic antiphospholipid syndrome-related microangiopathy (Espinosa et al., 2011).

Mesenchymal stem cells (MSC) are stromally derived progenitor cells, readily obtained from bone marrow, synovial, or adipose tissue, that can differentiate to all mesenchymal lineages, such as cartilage, bone, adipose tissue, or muscle. Most interestingly, MSCs display negative immunomodulatory effects *in vitro*. They suppress lymphocyte proliferation and antibody production, through partially unravelled mechanisms, involving cell-to-cell contacts and soluble factors. Their immunosuppressive properties have prompted preclinical research in animal models of autoimmune diseases, the most promising results being obtained in experimental autoimmune encephalomyelitis, a model of multiple sclerosis. Whether a similar approach can be applied to patients suffering

from autoimmune diseases is currently under investigation (Uccelli et al., 2011). Over 1000 humans have received MSCs (mostly allogeneic; MSCs are immunoprivileged) for various diseases, such as graft-versus-host disease, without noticeable adverse events. Intriguing positive results of two uncontrolled trials performed in LN patients have been published (Sun et al., 2010; Liang et al., 2010) but need to be confirmed in a proper randomized trial.

Duration of immunosuppressive therapy

Although we lack prospective trials specifically aimed at comparing different lengths of maintenance regimens, the very high renal relapse rate (35% in most long-term series) favours prolonged immunosuppression. In a small but interesting withdrawal study performed in LN patients, an inverse correlation was found between the length of treatment and remission before withdrawal and the risk of relapse (Moroni et al., 2006). Interestingly, in Europe, current practice is to keep patients long term on AZA/MMF and on low-dose GC. The possibility that the excellent long-term renal outcome observed in the Euro-Lupus Nephritis Trial (8% ESRD at 10 years) (Houssiau et al., 2010) is linked to prolonged immunosuppression is far from proven, yet not too far-fetched.

Treatment of membranous lupus nephritis

Membranous (class V) LN is characterized by subepithelial immune deposits. When associated with 'proliferative' disease, namely class III (focal) or class IV (diffuse) LN, most physicians consider that the presence of proliferative lesions guides therapy. However, some patients suffer from isolate membranous LN, either as the initial manifestation of renal involvement, or later in its evolution. Thus, patients treated for proliferative disease sometimes shift to membranous LN, unmasked by a control kidney biopsy performed because of persisting proteinuria despite immunosuppression and improvement in serological activity. Membranous LN should not be considered as a benign disease, since a substantial proportion of patients will develop ESRD. Moreover, persistent proteinuria or nephrotic syndrome may per se cause significant comorbidities, such as thromboembolic events and increased cardiovascular risk.

Treatment of pure membranous LN does not differ much from that of idiopathic membranous nephropathy, the more so as few controlled trials have specifically addressed this issue in LN. A distinction must be made between patients with low-grade or high-grade proteinuria. Thus, in patients with low-grade proteinuria, a 'watchful waiting' approach with optimal blockade of the angiotensin–renin–aldosterone system is mostly appropriate, together with lifestyle changes. In patients with high proteinuria, immunosuppressants are usually added, combining GC and one of the following drugs: CY, MMF, AZA, or calcineurin inhibitors. In a controlled trial performed at the NIH, 42 patients with pure membranous LN received (a) ciclosporin and alternate-day GC; or (b) alternate-month IV CY for 6 doses and alternate-day GC; or (c) alternate-day GC alone. Regimens (a) and (b) were both found superior to (c) at 1 year, but, interestingly, while the effect was prompter with calcineurin inhibitors, relapses were more frequent than in the CY group (Austin et al., 2009). A subset analysis performed in the 84 pure membranous LN cases randomized in two large, recent, randomized LN trials comparing MMF and IV CY (Ginzler et al., 2005; Appel et al., 2009) revealed that both drugs were equally effective at 24 weeks (Radhakrishnan et al., 2010). MMF is therefore a reasonable first-choice therapy in class V LN.

Treatment of lupus nephritis in pregnancy

Pregnant lupus patients with a past history of nephritis run a higher risk of maternal and fetal complications, such as hypertension, preeclampsia, eclampsia, fetal loss, intrauterine growth retardation, premature birth, etc. (Smyth et al., 2010). Patients on maintenance immunosuppression and whose renal disease is under control should not change their treatment, except if they take drugs that are forbidden during pregnancy, such as MMF, methotrexate, ACEIs, non-steroidal anti-inflammatory drugs, or diuretics. Withdrawal of ACEIs can lead to increased proteinuria, which is already the case in pregnant LN patients due to increased GFR. Shift from MMF to AZA may be associated with a renal flare. By contrast, steroids, AZA and HCQ can be (should be!) maintained throughout pregnancy to prevent lupus flares. Pregnant lupus patients with active renal disease (either because they become pregnant promptly after onset of renal involvement or because their LN started while pregnant) require very careful monitoring and should be immunosuppressed with GC (including, if needed, IV pulses of MP which can be safely administered during pregnancy). AZA can be added as a steroid-sparing agent, instead of IV CY or MMF. In exceptional cases, the latter drugs can be prescribed after the first trimester of pregnancy, when organogenesis is complete. Medical termination of pregnancy can be discussed in selected cases. Hypertension should be treated with alpha-methyldopa, calcium channel blockers, or labetalol. The presence of an antiphospholipid syndrome may further interfere with lupus pregnancy, as discussed elsewhere. There is some evidence from the non-lupus literature that low-dose aspirin prevents pre-eclampsia (Askie et al., 2007), thereby explaining why this treatment is recommended in pregnant lupus patients with a history of LN (Bertsias et al., 2012).

Targeted therapies

Fig. 163.1 depicts a simplified view of the pathophysiology of SLE and indicates some of the many potential targets for biologic therapy in LN. Not all have been (or will be) investigated. Sadly, none of those tested so far in randomized controlled trial, namely abetimus, rituximab (RTX; anti-CD20 mAb), ocrelizumab (OCR; humanized anti-CD20 mAb), and abatacept (CTLA4-Ig), were found to be superior to standard of care (SOC).

The LUNAR (*Lu*pus *N*ephritis *A*ssessment with *R*ituximab) trial was a phase 3 randomized, double-blinded, placebo-controlled, multicentre study including class III or IV LN patients (Rovin et al., 2012). All patients received GC and MMF as background therapy. RTX (two infusions of 1 g, 15 days apart with re-treatment 6 months later) or placebo was given as add-on treatment. The primary outcome, that is, the proportion of patients achieving complete or partial renal response at week 52, was met by 57% and 46% of the patients randomized in the RTX and placebo group, respectively. This difference was not statistically significant. A trend in favour of RTX was noted in African Americans (70% vs 45%).

The BELONG trial was aimed at testing the efficacy and safety of OCR in patients with active ISN/RPS class III or IV LN treated with SOC consisting of GC and either MMF or the Euro-Lupus regimen, as selected by investigators (Mysler et al., 2013). The study

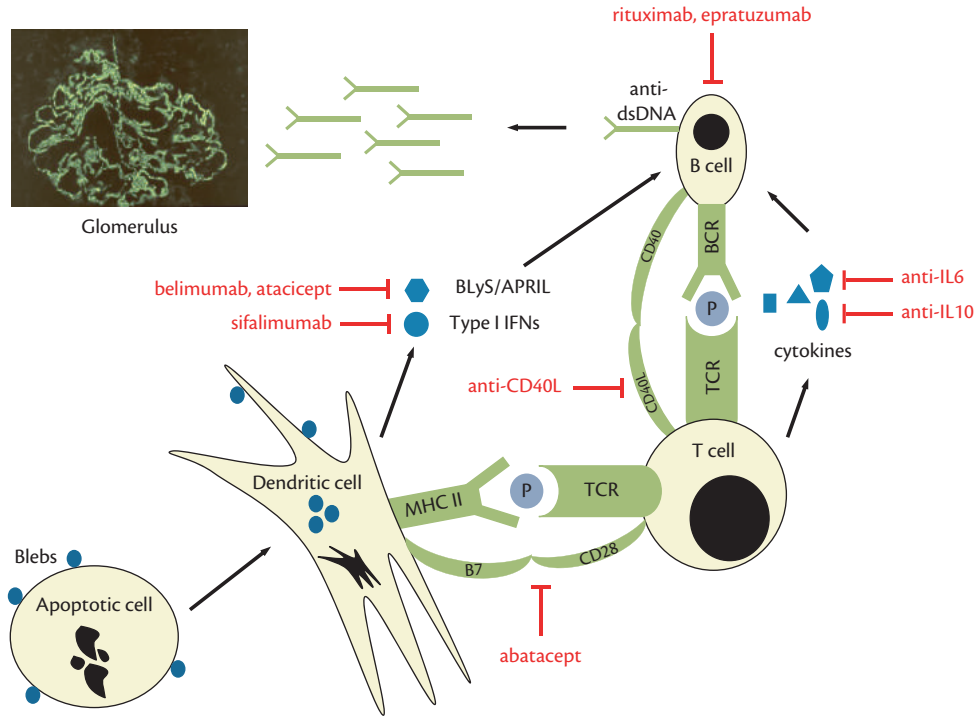

Fig. 163.1 Targeted therapies in lupus nephritis. Clearance of apoptotic bodies is impaired in systemic lupus erythematosus and autoantigen-containing apoptotic material is processed by dendritic cells (instead of phagocytes) and presented to T-helper cells, which in turn cooperate with B-cells, in the presence of optimal co-stimulation (through CD40-CD40L and CD28-B7.1/B7.2). Cytokines are produced by dendritic cells and activated T-helper cells, and further stimulate B-cells to produce high-affinity anti-dsDNA antibodies, which bind to glomerular structures, such as the basement membrane, and initiate glomerulonephritis. Potential targeted therapies are indicated in red.

was terminated early, after complete enrolment, because an interim analysis revealed safety concerns. Thus, serious infections, that is, requiring IV antibiotics, were twice as common in the 400 mg OCR group (surprisingly not in the 1000 mg OCR group) compared to placebo. Intriguingly, this was the case only in the subgroup of patients given MMF as SOC background therapy (not on Euro-Lupus background).

The negativity of these two B-cell blockade LN trials, together with the negative results of the EXPLORER (*Explor*atory Phase II/III SLE *E*valuation of *R*ituximab) trial performed in non-renal lupus patients (Merrill et al., 2010), contrasts with the clinical practice of experienced physicians who have been successfully using RTX in LN cases who had failed on conventional therapy, thereby raising concerns about the design of the two RTX LN studies. Did concomitant therapy (with high doses of GC and other immunosuppressants) mask the effects of RTX? Were the trials too short term to unmask the benefits of RTX? Were the doses of RTX appropriate? Should RTX be prescribed only in a subset of early refractory LN cases not responding to SOC after 3–6 months? These are some of the many questions that should be addressed before RTX be considered a falling star! In this respect, the recent positive result of EMBLEM (Wallace et al., 2013), a short-term phase 2 epratuzumab (anti-CD22) trial performed in lupus patients without severe renal/central nervous system involvement, further stress the potential value of a B-cell blockade approach in lupus.

Two randomized trials were designed in order to test the efficacy of abatacept as add-on induction therapy for LN. The molecule, also called CTLA4Ig, is a selective T-cell co-stimulation modulator that binds to B7 (expressed on antigen-presenting cells) and thereby blocks its interaction with CD28, expressed on T cells, preventing T-cell activation. An industry-sponsored trial testing abatacept on a GC and MMF background was recently reported to be inconclusive (Furie et al., 2014), although other post hoc definitions of the primary endpoint may lead to different conclusions (Wofsy et al., 2013). We now eagerly await the results of the ACCESS trial (*A*batacept and *C*yclophosphamide *C*ombination: *E*fficacy and *S*afety *S*tudy), a study sponsored by the Immune Tolerance Network and the National Institute for Allergy and Infectious Diseases, aimed at comparing abatacept and placebo on a Euro-Lupus regimen background.

Other biologics will most likely be tested in LN. The very recent approval US Food and Drug Administration of belimumab for the treatment of moderately severe lupus, after the success of two phase 3 pivotal trials performed in non-renal lupus patients (Navarra et al., 2011), will likely trigger a proper LN trial with the drug. Anti-CD22 epratuzumab, anti-IL6, anti-IL6R, and anti-type I IFNs are also likely to be investigated.

Optimal multidisciplinary care

Long-term prognosis of LN patients relies upon excellent daily care given in specialized centres aimed at controlling all aspects of such a complex disease. In other words, until a wonder drug becomes available, most of the differences in outcome may well be related to the quality of care, as in many other chronic diseases.

At diagnosis, physicians, dedicated nurse practitioners, and patients' associations should provide adequate educational material that replaces inaccurate and mostly frightening uncontrolled

websites and forums, favouring misconceptions. Follow-up must be obsessional, following a standardized scheme and directly involving patients.

Since non-adherence to pharmacological therapy is one of the major reasons for treatment failure, it should be discussed with the patient from the very first visit and always suspected in 'refractory' cases. Interestingly, measurements of whole-blood hydroxychloroquine (HCQ) titres may help in detecting poor adherence. The drug is (should be) prescribed in all lupus patients, including those who suffer from LN, based on its proven ability to prevent lupus flares and its excellent toxicity profile. Due to its long elimination half-life, HCQ accumulates in the blood in patients, at least in those taking the drug on a regular basis. Those who swallow only a few pills the days before the visit—a phenomenon known as 'white coat compliance'—have very low HCQ whole-blood titres and can therefore be unmasked. Using this tool as an index of poor adherence to therapy, a recent study could demonstrate that non-compliant lupus patients experienced more lupus flares, thereby further demonstrating the pivotal role of HCQ in the management of SLE (Costedoat-Chalumeau et al., 2007).

As in other chronic renal diseases (see KDOQI (Kidney Disease Outcomes Quality Initiative) and KDIGO (Kidney

Table 163.1 Standard immunosuppressive drugs for induction and maintenance therapy of ISN/RPS class III/IV/V lupus nephritis

Drug	Route	Dose	Induction	Maintenance	Pregnancy	Side effects	Monitoring
Glucocorticoids	Oral	0.5–1.0 mg/kg/day prefer 0.5 mg/kg/day	**Required** Combined to another IS	Advised Low dose (5–7.5 mg/day)	Allowed	Cushingoid features Infections Etc.	Glucose BMD K
	IV	500–1000 mg MP	Advised 3 consecutive days	Optional (monthly to 3-monthly) Consider in non-compliant patients	Allowed	Cushingoid features Infections Etc.	Glucose BMD K
Cyclophosphamide	Oral	2 mg/kg/day	Only for highly selected severe cases	No	Forbidden	Gonadal failure Cytopenia GI intolerance Haemorrhagic cystitis Bladder cancer	FBC Liver tests
	IV NIH	500–1000 mg/m^2 6-monthly pulses dose adjusted (WBC nadir) Mesna® advised	Reasonable *1st choice*	No	Forbidden	Gonadal failure Cytopenia GI intolerance	FBC Liver tests
	IV EL	500 mg fixed dose 6-fortnightly pulses	Reasonable *1st choice*	Not applicable	Forbidden		FBC Liver tests
Mycophenolate mofetil	Oral	1–3 g/day Target 2–3 g/day	Reasonable *1st choice* Preferred for non-Caucasians, non-Asians	Reasonable *1st choice*	Forbidden	Diarrhoea Cytopenia Hepatitis Teratogenicity	FBC Liver tests
Azathioprine	Oral	1.0–2.5 mg/kg/day Target 2.0–2.5 mg/kg/day	Not 1st choice	Reasonable *1st choice*	Allowed	Nausea Cytopenia Hepatitis Hypersensitivity	TPMT genotype FBC Liver tests
Calcineurin inhibitors	Oral	Ciclosporin: 2.5–3.0 mg/kg/day Tacrolimus: 0.05 mg/kg/day	Not 1st choice	Selected cases Pure membranous lupus nephritis	Allowed	Nephrotoxicity Hypertension Hypertrichosis Hyperlipidaemia Tremor	GFR BP Lipids Glucose
Plasma exchanges	NA	2–4 L/session twice weekly, then weekly	Selected cases	No	Difficult	Infections	IgG titres

BMD = bone mineral density measurement; BP = blood pressure; EL = Euro Lupus; FBC = full blood cells count; GFR = glomerular filtration rate; GI = gastrointestinal; IS = immunosuppressant; IV = intravenous; K = potassium; MP = methylprednisolone; NA = not applicable; NIH = National Institutes of Health; TPMT = thiopurine methyltransferase; WBC = white blood cells count.

Disease: Improving Global Outcomes) guidelines) blood pressure must be tightly controlled and maximal systolic/diastolic values should not exceed 120/80 mm Hg. Antiproteinurics should be prescribed in all cases of LN, if needed by combining ACEIs, ARBs, and loop diuretics.

Pivotal data by the Toronto group first pointed to a bimodal mortality pattern in lupus patients, with an early peak related to lupus itself and its therapy, and a second due to cardiovascular disease (Urowitz et al., 1976). This observation was largely confirmed by the formal demonstration of accelerated atheroma in lupus patients, as assessed by carotid Doppler studies (plaques and intima–media thickness) (Roman et al., 2003) and by coronary computed tomography scan studies (calcifications) (Asanuma et al., 2003). In this respect, control of other cardiovascular risk factors is of the utmost importance, such as smoking avoidance, weight, exercise, and cholesterol levels. As for diabetes mellitus, we set the cut-off for treatment with a cholesterol-lowering drug as low as 115 mg/dL of low-density lipoprotein cholesterol.

Numerous studies have demonstrated that many lupus patients suffer from GC-induced osteoporosis (Houssiau et al., 1996) that should always be prevented by calcium salts and vitamin D_3 supplements and, in selected cases, by bisphosphonates. All lupus patients should be immunized against *Streptococcus pneumoniae* (Naveau and Houssiau, 2005), given the incidence and severity of the infections caused by this pathogen. The higher prevalence of uterine cervix carcinoma and human papillomavirus infection in lupus patients compared to a control population (Tam et al., 2004) strongly argues in favour of early immunization in teenage lupus patients.

Renal replacement therapy and transplantation

For the 10–30% of LN patients who develop ESRD, RRT or RT is the only remaining option. In this respect, in patients with severe sustained renal impairment and major chronic glomerular and/or interstitial changes on renal biopsy, it may be wiser to opt for RRT rather than to introduce an additional immunosuppressant or a new biologic agent, to avoid unnecessary infectious episodes. This attitude is all the more appropriate since survival of lupus patients on haemodialysis (HD) is by and large comparable to the general population (Mojcik and Klippel, 1996). ESRD lupus patients still on immunosuppressive therapy experience significantly more peritonitis while on continuous ambulatory peritoneal dialysis (CAPD) compared to non-lupus patients (Huang et al., 2001). For this very reason, HD may be preferred to CAPD in patients with LN still immunosuppressed (Rietveld and Berden, 2008), although this remains debated. Disease activity and serum markers usually decrease in lupus patients with ESRD and remain so on RRT, with some exceptions that require reintroduction of specific therapy. Lupus patients with ESRD, when in clinical remission, are good candidates for RT, as suggested by graft and patient survival rates, again comparable to other groups of patients (Bunnapradist et al., 2006). Pre-emptive graft with a living donor reduces the waiting time for RT and is therefore likely to improve survival rates. Recurrence of lupus in the transplanted kidney is a surprisingly low event (< 5%) and is probably prevented by antirejection immunosuppressive therapy. The presence of an antiphospholipid syndrome may complicate RRT (thromboembolic events) and RT (graft thrombotic microangiopathy). Much attention should be paid to cardiovascular pre-transplant screening.

Synopsis

Table 163.1 tentatively summarizes the respective indications of the most commonly used immunosuppressants, as well as some more practical points. Box 163.1 serves as an executive summary by stressing the key points to consider when deciding treatment in a LN patient. Both tables do not strive for completeness, nor are they intended to serve as recommendations, such as those edited by the European League Against Rheumatism and the European Renal Association-European Dialysis and Transplant Association (Bertsias et al., 2012) or by the American College of Rheumatology (Hahn et al., 2012). Rather, they should highlight the critical issues that must be taken into account before making a decision at the bedside. Not unexpectedly, not all LN patients respond to the same first-line immunosuppression and we cannot predict who is going

Box 163.1 Key points to consider when treating LN[a]

- Activity and chronicity indices on renal biopsy (and not only the ISN/RPS class) should be taken into account before making a treatment decision
- Whenever possible, use moderate doses of oral glucocorticoids (e.g. 0.5 mg/kg/day) and start with IV methylprednisolone therapy (e.g. 500–1000 mg on 3 consecutive days) to reduce oral glucocorticoid starting dose
- The choice for induction treatment is mostly between intravenous cyclophosphamide and mycophenolate mofetil
- Mycophenolate mofetil is at least equivalent to cyclophosphamide to induce a renal response
- If cyclophosphamide is chosen, prefer an IV regimen, either the Euro-Lupus regimen (6 × 500 mg every fortnight) or the NIH 6 months induction regimen
- Treatment shift (from IV cyclophosphamide to mycophenolate mofetil or vice versa) is advisable in case of poor response after 3–6 months of induction therapy
- The choice for maintenance treatment is mostly between mycophenolate mofetil and azathioprine, in combination with low-dose glucocorticoids
- Given the high relapse rate, long-term maintenance treatment is advisable
- Optimal multidisciplinary care is pivotal
- In some patients with severe sustained renal impairment and major chronic glomerular/interstitial changes on renal biopsy, it may be wiser to opt for renal replacement therapy (when needed) and to step down immunosuppressive therapy, instead of introducing yet another potentially toxic drug
- Patients' specificities (e. g. ethnicity) and perspectives (e.g. pregnancy plans) need to be taken into account.

[a]These key points apply mainly for proliferative LN (ISN/RPS class III ± V; class IV ± V).

to respond to which drug. One way to overcome this shortcoming, and to limit the consequences of a 'trial and error' approach, is to evaluate the early response to therapy and to shift to another treatment in case of unsatisfactory response.

Despite the many progresses described in this chapter, several needs remain unmet. How can we identify patients at baseline who run the highest risk for ESRD? Which biomarkers will help us to predict renal relapses? What is the minimal GC dose? Should we aim at inducing a complete renal remission? Should we use combination therapy? What is the place of anti-B-cell therapy? How long should patients be treated? All of these issues clearly deserve further evidence-based data.

References

Anderka, M. T., Lin, A. E., Abuelo, D. N., *et al.* (2009). Reviewing the evidence for mycophenolate mofetil as a new teratogen: case report and review of the literature. *Am J Med Genet*, 149A, 1241–8.

Appel, G. B., Contreras, G., Dooley, M. A., *et al.* (2009). Mycophenolate mofetil versus cyclophosphamide for induction treatment of lupus nephritis. *J Am Soc Nephrol*, 20, 1103–12.

Asanuma, Y., Oeser, A., Shintani, A. K., *et al.* (2003). Premature coronary-artery atherosclerosis in systemic lupus erythematosus. *N Engl J Med*, 349, 2407–15.

Askie, L. M., Duley, L., Henderson-Smart, D. J., *et al.* (2007). Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. *Lancet*, 369, 1791–8.

Austin, H. A. 3rd, Illei, G. G., Braun, M. J., *et al.* (2009). Randomized, controlled trial of prednisone, cyclophosphamide, and cyclosporine in lupus membranous nephropathy. *J Am Soc Nephrol*, 20, 901–11.

Austin, H. A. 3rd, Klippel, J. H., Balow, J. E., *et al.* (1986). Therapy of lupus nephritis. Controlled trial of prednisone and cytotoxic drugs. *N Engl J Med*, 314, 614–19.

Bertsias, G. K., Tektonidou, M., Amoura, Z., *et al.* (2012). Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheumat Dis*, 71, 1771–82.

Boumpas, D. T., Austin, H. A. 3rd, Vaughn, E. M., *et al.* (1992). Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. *Lancet*, 340, 741–5.

Bunnapradist, S., Chung, P., Peng, A., *et al.* (2006). Outcomes of renal transplantation for recipients with lupus nephritis: analysis of the Organ Procurement and Transplantation Network database. *Transplantation*, 82, 612–18.

Cervera, R., Khamashta, M. A., Font, J., *et al.* (2003). Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. *Medicine (Baltimore)*, 82, 299–308.

Contreras, G., Pardo, V., Leclercq, B., *et al.* (2004). Sequential therapies for proliferative lupus nephritis. *N Engl J Med*, 350, 971–80.

Condon, M.B., Ashby, D., Pepper, R., *et al.* (2013). Prospective observational single center cohort study to evaluate the effectiveness of treating lupus nephritis with rituximab and mycophenolate mofetil but no oral steroids. *Ann Rheumat Dis*, 72(8), 1280–6.

Costenbader, K. H., Desai, A., Alarcon, G. S., *et al.* (2011). Trends in the incidence, demographics, and outcomes of end-stage renal disease due to lupus nephritis in the US from 1995 to 2006. *Arthritis Rheum*, 63, 1681–8.

Donadio, J. V. Jr., Holley, K. E., Wagoner, R. D., *et al.* (1972). Treatment of lupus nephritis with prednisone and combined prednisone and azathioprine. *Ann Intern Med*, 77, 829–35.

Dooley, M. A., Jayne, D., Ginzler, E. M., *et al.* (2011). Mycophenolate versus azathioprine as maintenance for lupus nephritis. *N Engl J Med*, 365, 1886–95.

Espinosa, G., Berman, H., and Cervera, R. (2011). Management of refractory cases of catastrophic antiphospholipid syndrome. *Autoimmune Rev*, 10, 664–8.

Ford, L. T. and Berg, J. D. (2010). Thiopurine S-methyltransferase (TPMT) assessment prior to starting thiopurine drug treatment; a pharmacogenomic test whose time has come. *J Clin Pathol*, 63, 288–95.

Furie, R., Nicholls, K., Cheng, T. -T., *et al.* (2014). Efficacy and safety of abatacept in lupus nephritis: a 12-month, randomized, double-blind study. *Arthritis Rheumatol*, 66(2), 379–89.

Ginzler, E. M., Dooley, M. A., Aranow, C., *et al.* (2005). Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis. *N Engl J Med*, 353, 2219–28.

Gourley, M. F., Austin, H. A. 3rd, Scott, D., *et al.* (1996). Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis. A randomized, controlled trial. *Ann Intern Med*, 125, 549–57.

Grootscholten, C., Ligtenberg, G., Hagen, E. C., *et al.* (2006). Azathioprine/methylprednisolone versus cyclophosphamide in proliferative lupus nephritis. A randomized controlled trial. *Kidney Int*, 70, 732–42.

Hahn, B. H., McMahon, M. A., Wilkinson, A., *et al.* (2012). American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res*, 64, 797–808.

Houssiau, F. A., D'Cruz, D., Sangle, S., *et al.* (2010b). Azathioprine versus mycophenolate mofetil for long-term immunosuppression in lupus nephritis: results from the MAINTAIN Nephritis Trial. *Ann Rheumat Dis*, 69, 2083–9.

Houssiau, F. A., Lefebvre, S., Depresseux, G., *et al.* (1996). Trabecular and cortical bone loss in systemic lupus erythematosus. *Br J Rheumatol*, 35, 244–7.

Houssiau, F. A., Vasconcelos, C., D'Cruz, D., *et al.* (2002). Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high dose intravenous cyclophosphamide. *Arthritis Rheum*, 46, 2121–31.

Houssiau, F. A., Vasconcelos, C., D'Cruz, D., *et al.* (2004). Early response to immunosuppressive therapy predicts good renal outcome in lupus nephritis: lessons from long-term followup of patients in the Euro-Lupus Nephritis Trial. *Arthritis Rheum*, 50, 3934–40.

Houssiau, F. A., Vasconcelos, C., D'Cruz, D., *et al.* (2010). The 10-year follow-up data of the Euro-Lupus Nephritis Trial comparing low-dose and high-dose intravenous cyclophosphamide. *Ann Rheumat Dis*, 69, 61–4.

Huang, J. W., Hung, K. Y., Yen, C. J., *et al.* (2001). Systemic lupus erythematosus and peritoneal dialysis: outcomes and infectious complications. *Periton Dial Int*, 21, 143–7.

Lee, Y. H., Lee, H. S., Choi, S. J., *et al.* (2011). Efficacy and safety of tacrolimus therapy for lupus nephritis: a systematic review of clinical trials. *Lupus*, 20, 636–40.

Lewis, E. J., Hunsicker, L. G., Lan, S. P., *et al.* (1992). A controlled trial of plasmapheresis therapy in severe lupus nephritis. The Lupus Nephritis Collaborative Study Group. *N Engl J Med*, 326, 1373–9.

Liang, J., Zhang, H., Bua, B., *et al.* (2010). Allogenic mesenchymal stem cells transplantation in refractory systemic lupus erythematosus: a pilot clinical study. *Ann Rheumat Dis*, 69, 1423–9.

Merrill, J. T., Neuwelt, C. M., Wallace, D. J., *et al.* (2010). Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum*, 62, 222–33.

Mojcik, C. F. and Klippel, J. H. (1996). End-stage renal disease and systemic lupus erythematosus. *Am J Med*, 101, 100–7.

Moroni, G., Gallelli, B., Quaglini, S., *et al.* (2006). Withdrawal of therapy in patients with proliferative lupus nephritis: long-term follow-up. *Nephrol Dial Transplant*, 21, 1541–8.

Moroni, G., Doria, A., and Ponticelli, C. (2009). Cyclosporine (CsA) in lupus nephritis: assessing the evidence. *Nephrol Dial Transplant*, 24, 15–20.

Mysler, E. F., Spindler, A. J., Guzman, R., *et al.* (2013). Efficacy and safety of ocrelizumab in active proliferative lupus nephritis: results from the

randomized double-blind Phase III BELONG study. *Arthritis Rheum*, 65(9), 2368–79.
Navarra, S. V., Guzman, R. M., Gallacher, A. E., *et al.* (2011). Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomized, placebo-controlled, phase 3 trial. *Lancet*, 377, 721–31.
Naveau, C. and Houssiau, F. A. (2005). Pneumococcal sepsis in patients with systemic lupus erythematosus. *Lupus*, 14, 903–6.
Nguyen, T., Vacek, P. M., O'Neil P., *et al.* (2009). Mutagenicity and potential carcinogenicity of thiopurine treatment in patients with inflammatory bowel disease. *Cancer Res*, 69, 7004–12.
Radhakrishnan, J., Moutzouris, D. A., Ginzler, E. M., *et al.* (2010). Mycophenolate mofetil and intravenous cyclophosphamide are similar as induction therapy for class V lupus nephritis. *Kidney Int*, 77, 152–60.
Ramsey-Goldman, R. and Schilling, E. (1997). Immunosuppressive drug use during pregnancy. *Rheum Dis Clin North Am*, 23, 149–67.
Rietveld, A. and Berden, J. H. (2008). Renal replacement therapy in lupus nephritis. *Nephrol Dial Transplant*, 23, 3056–60.
Roman, M. J., Shanker, B. A., Davis, A., *et al.* (2003). Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *N Engl J Med*, 349, 2399–406.
Rovin, B. H., Furie, R., Latinis, K., *et al.* (2012). Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab Study. *Arthritis Rheum*, 64, 1215–26.
Rovin, B. H., McKinley, A. M., and Birmingham, D. J. (2009). Can we personalize treatment for kidney diseases? *Clin J Am Soc Nephrol*, 4, 1670–6.
Smyth, A., Oliveira, G. H., Lahr, B. D., *et al.* (2010). A systematic review and meta-analysis of pregnancy outcomes in patients with systemic lupus erythematosus and lupus nephritis. *Clin J Am Soc Nephrol*, 5, 2060–8.
Stoenoiu, M., Aydin, S., Tektonidou, M., *et al.* (2012). Repeat kidney biopsies fail to detect differences between azathioprine and mycophenolate mofetil maintenance therapy for lupus nephritis: data from the MAINTAIN Nephritis Trial. *Nephrol Dial Transplant*, 27, 1924–30.
Sun, L., Wang, D., Liang, J., *et al.* (2010). Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. *Arthritis Rheum*, 62, 2467–75.
Tam, L. S., Chan, A. Y., Chan, P. K., *et al.* (2004). Increased prevalence of squamous intraepithelial lesions in systemic lupus erythematosus: association with human papillomavirus infection. *Arthritis Rheum*, 50, 3619–25.
Uccelli, A., Laroni, A., and Freedman, M. S. (2011). Mesenchymal stem cells for the treatment of multiple sclerosis and other neurological diseases. *Lancet Neurology*, 10, 649–56.
Urowitz, M. B., Bookman, A. A., Koehler, B. E., *et al.* (1976). The bimodal mortality pattern of systemic lupus erythematosus. *Am J Med*, 60, 221–5.
Wallace, D. J., Kalunian, K., Petri, M. A., *et al.* (2013). Efficacy and safety of epratuzumab in patients with moderate/severe systemic lupus erythematosus: results from EMBLEM, a phase IIb, randomized, double-blind, placebo-controlled, multicenter study. *Ann Rheum Dis*, 73(1), 183–90
Walsh, M., Solomons, N., Lisk, L., *et al.* (2013). Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis with poor kidney function: a subgroup analysis of the Asprava Lupus Management Study. *Am J Kidney Dis*, 61, 710–15.
Wofsy, D., Hillson, J. L. and Diamond, B. (2013). Comparison of alternative primary outcome measures for use in a lupus nephritis trial. *Arthritis Rheum*, 65(6), 1586–91.

CHAPTER 164

The patient with antiphospholipid syndrome with or without lupus

Bassam Alchi and David Jayne

Introduction

The antiphospholipid syndrome (APS) (Box 164.1) was first described by Hughes in the mid 1980s as a disorder of hypercoagulability in association with antiphospholipid antibodies (aPL), reported in the context of systemic lupus erythematosus (SLE) (Hughes, 1983). APS also occurs, less frequently, in the absence of associated autoimmune disease: 'primary APS'. In the past two decades, a variety of immunologically mediated thrombotic events related to almost every organ system have been identified as features of this syndrome (Ruiz-Irastorza et al., 2010). APS is increasingly recognized as an important cause of renal injury, and nephrologists are more frequently involved in managing patients with APS, whether it is primary, SLE-related, in the severe catastrophic antiphospholipid syndrome (CAPS), before and after dialysis onset, or at transplantation (Alchi et al., 2010).

Antiphospholipid antibodies

aPL are a heterogeneous group of antibodies observed in a range of pathological conditions, as well as in healthy populations (Biggioggero and Meroni, 2010). They play a crucial role in the pathogenesis of APS (Box 164.2); however, not all patients with these antibodies develop clinical features of APS, for example, aPL are found in approximately 30–40% of patients with SLE, but only 10% have APS. A 'two-hit hypothesis' has been suggested to explain the clinical observation that thrombotic events occur only occasionally, in spite of the persistent presence of aPL. According to this principle, the antibody (representing the first hit) induces a thrombophilic state, but clotting takes place only in the presence of another thrombophilic condition (the second hit), for example, infection or trauma (Willis et al., 2012).

Antibodies to cardiolipin (aCL) and beta-2 glycoprotein 1 (β_2GP1) of immunoglobulin (Ig)-G and IgM isotype are routinely measured in serum by solid-phase immunoassays. Lupus anticoagulant (LA) is suggested by a prolonged activated partial thromboplastin time and confirmed by prolongation of the diluted Russell viper venom test indicating the presence of a transferable inhibitor of coagulation. Anticoagulation with oral vitamin K antagonists interferes with and can invalidate the results of LA. Taipan snake venom time assay is preferably used to diagnose LA in patients receiving anticoagulants (Parmar et al., 2009).

It is unclear which patients with aPL will develop thrombosis. In general, LA are more specific for APS, whereas aCL are more sensitive. The association between aPL and thrombosis is stronger with LA than with aCL. In a meta-analysis of 25 studies involving 7000 patients, the odd ratios for thrombosis was 11.0 for LA and 1.6 for aCL (Galli 2003). Moreover, LA for which the prolongation of clotting times is dependent on the presence of β_2GP1 show much stronger association with thrombosis (odds ratio, 42.3) than do LA that are independent of β_2GP1 (odds ratio, 1.6) (de Laat et al., 2004).

Renal manifestations of antiphospholipid syndrome

The most common renal presentation of APS, with or without lupus, is with hypertension, proteinuria, active urinary sediment, and acute or progressive renal dysfunction. Hypertension may be severe, with some patients presenting with hypertensive emergencies. Nephrotic syndrome occurs in one-third of patients with SLE-related APS, while it is rare in primary APS. Any part of renal vasculature can be affected in APS, and the clinical presentation depends on the size of blood vessel involved (Nochy et al., 1999; Amigo, 2006; D'Cruz, 2009). In a survey of 160 patients with primary APS, 9% had renal abnormalities, all with proteinuria. Renal biopsy revealed antiphospholipid nephropathy (APSN), membranous and proliferative glomerulonephritis, and the subgroup with renal disease were more likely to be LA positive and to have hypocomplementaemia (Sinico et al., 2010).

Renal artery lesions

APS is a unique, non-traditional cause of renal artery stenosis (RAS) with important clinical consequences. It may manifest in multiple ways ranging from renal infarction to ischaemic acute kidney injury to slowly progressive ischaemic chronic renal failure to renovascular disease. Using magnetic resonance angiography, 26% of aPL-positive patients with poorly controlled hypertension were found to have RAS as compared to 8% of young (≤50 years) hypertensive controls and 3% of healthy potential kidney donors (Sangle et al., 2003). Two patterns of stenotic lesions have been described in APS. The more common pattern is characterized by smooth, well-delineated, and often non-critical stenoses in the mid

Box 164.1 Renal manifestations of antiphospholipid syndrome

- Vascular:
  - Renal artery stenosis
  - Renal vein thrombosis
  - Antiphospholipid syndrome nephropathy
  - Antiphospholipid syndrome associated with primary glomerulonephritis
- End-stage renal disease:
  - Dialysis access thrombosis
- Transplant:
  - Allograft thrombosis.

portion of the renal artery, quite distinct from either fibromuscular dysplasia or atherosclerosis. The less common pattern is similar to atherosclerotic lesions situated proximally and occasionally involving the aorta. The stenotic lesions often have both thrombotic and a reactive or proliferative components, with intimal mucoid thickening, subendothelial fibrosis, and medial hyperplasia. Renal artery imaging should be considered as RAS/thrombosis may respond to anticoagulation (Sangle et al., 2005).

Renal infarction

Renal infarction results from occlusive lesions in smaller diameter intraparenchymal vessels, caused by *in situ* thrombosis or emboli from a pre-existing proximal arterial or cardiac lesion. Patients with renal infarction present with flank pain, severe hypertension, and/or renal dysfunction. Some may have multiple, often serious, thrombotic episodes, and many have multiple infarctions in the renal cortex. Associated infarction of the adrenal glands is also seen. The renal pathology in these patients is characterized by glomerular ischaemia, tubular atrophy, and interstitial fibrosis.

Box 164.2 Proposed thrombotic mechanisms mediated by aPL

- Disruption of endogenous antithrombotic mechanisms:
  - Interference with natural anticoagulants
  - Disruption of annexin A5 shield
  - Activation of protein C resistance
  - Inhibition of fibrinolysis
- aPL antibody-mediated pro-coagulant/pro-inflammatory cell signalling
- Induction of endothelial surface adhesion molecules
- Induction of tissue factor expression on monocytes and endothelial cells
- Interference with apoptotic cell clearance:
  - Stimulation of platelet aggregation
- Complement activation.

Antiphospholipid nephropathy

APSN refers to the kidney damage caused by vaso-occlusive lesions in the glomeruli, arterioles, and/or interlobular arteries in patients with aPL. APSN vascular lesions may be acute, as in thrombotic microangiopathy (TMA), and/or chronic, such as arteriosclerosis, fibrous intimal hyperplasia, tubular thyroidisation, and focal cortical atrophy (Box 164.3). APSN has been described in patients with primary APS, SLE-related APS, and SLE/non-APS patients with aPL. The same histologic lesions, especially TMA, are also observed in patients with catastrophic APS (Tektonidou et al., 2008). APSN is not yet considered a defining lesion of APS (Miyakis et al., 2006), and its true incidence is probably underestimated, in part because of the frequent occurrence of thrombocytopenia and systemic hypertension, discouraging renal biopsy. Patients with APS have an increased risk of haemorrhage after renal biopsy related to arterial vasculopathy, and suspension of anticoagulation to permit a biopsy can be complicated by a thrombotic event (Chaib et al., 2007). Nevertheless, ASPN may occur in as many as 25% of patients with primary APS and 40% of SLE patients with positive aPL. Among aPL-positive SLE patients, ASPN was found in two-thirds of those with APS and in one-third of those without APS (Tektonidou et al., 2004). The pathogenesis of APSN cannot be fully explained by the occurrence of ischaemic events, but it may also result from direct effects of aPL on the glomerular microcirculation (Meroni et al., 2011). Both LA and aCL have been associated with the occurrence of APSN. IgG anti-β_2GP1 antibodies are associated with TMA occurring in lupus nephritis and with C4d deposition in the thrombotic lesions indicating a pathogenic role for these antibodies and complement (Shen et al., 2010). In contrast, IgM anti-β_2GP1 antibodies have been associated with a more benign course in lupus nephritis, suggesting a protective effect on vascular injury (Mehrani and Petri, 2011).

Clinically, APSN manifests with hypertension, acute or chronic renal dysfunction, and low-grade proteinuria. The renal pathology of APSN is characterized by glomerular capillary thrombosis with associated mesangiolysis, mesangial interposition, and duplication of glomerular basement membrane, without inflammatory cells or vascular immune deposits. These histological features resemble other forms of TMA such as HUS-TTP. Children with haemolytic-uraemic syndrome-thrombotic thrombocytopenic

Box 164.3 Definition of antiphospholipid syndrome nephropathy (Sydney International Consensus, 2006)

APSN is the coexistence of aPL along with the histopathologic detection of:

- Thrombotic microangiopathy involving both arterioles and glomerular capillaries (Fig. 164.1) *and/or*
- One or more of:
  - Fibrous intimal hyperplasia involving organized thrombi with or without recanalization (Fig. 164.2)
  - Fibrous and/or fibrocellular occlusions of arteries and arterioles
  - Focal cortical atrophy
  - Tubular thyroidization.

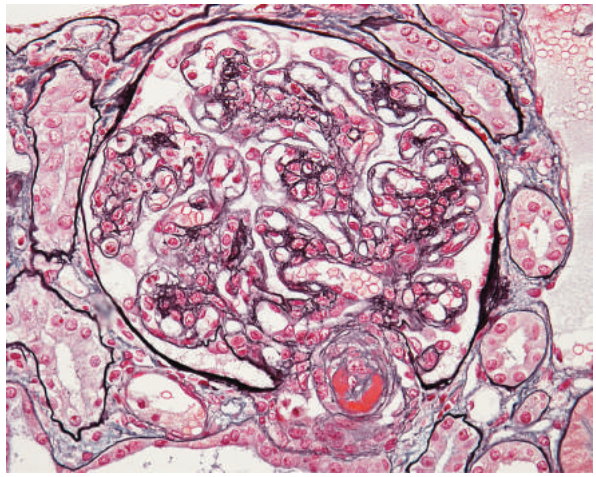

Fig. 164.1 Microangiopathic glomerulopathy. A glomerulus from a patient with positive antiphospholipid antibodies showing microangiopathic changes, including duplicated basement membranes, glomerular capillary thrombosis (seen in the afferent/efferent arteriole) with entrapment of fragmented erythrocytes. (Periodic acid–methenamine silver and Masson trichrome stain ×400.)
By courtesy of Dr Imai Naofumi, Department of Renal Pathology, Niigata University, Japan.

purpura (HUS-TTP) associated with autoantibodies to ADAMTS13 have gone on to develop SLE, lupus nephritis, and aPL, suggesting that this presentation is both a precursor of SLE and a form of APSN (Muscal et al., 2011).

In the chronic phase, arteriosclerosis is associated with fibrocellular intimal hyperplasia of the arteries and arterioles, with consequent lumen restriction and ischaemia. This leads to focal zones of cortical atrophy in the superficial cortex, in which atrophic tubules packed with eosinophilic casts (tubular thyroidization) are often present. Focal cortical atrophy causes depression of the contour of renal capsule, which may give a 'moth-eaten' appearance on renal imaging.

A non-thrombotic glomerular endothelial injury with a distinctive wrinkling and reduplication of the basement membrane has been associated with aPL (Griffiths et al., 2000). This type of injury is not included in the current APSN definition (Miyakis et al., 2006). APS has also been associated with membranous glomerulonephritis and other primary glomerulopathies (Fakhouri et al., 2003).

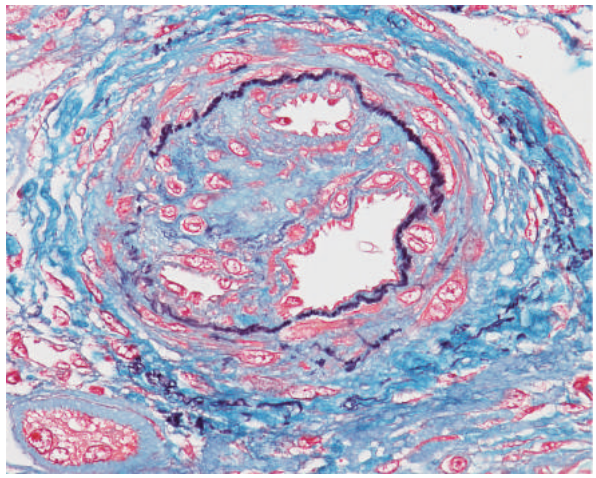

Fig. 164.2 Thrombotic arteriopathy. Renal arteriole from a patient with positive antiphospholipid antibodies showing intimal fibroplasia with re-canalized thrombus. (Elastica-Masson trichrome ×400.)
By courtesy of Dr Imai Naofumi, Department of Renal Pathology, Niigata University, Japan.

Renal vein thrombosis

APS, with or without SLE, may lead to thrombosis of the renal veins and inferior vena cava. Thus, in any patient with persistently positive aPL who develops sudden heavy proteinuria or acute deterioration of renal function, careful Doppler studies of the renal vasculature should be considered. Alternatively, contrast-enhanced computed tomography or magnetic resonance angiography can be used to provide the diagnosis.

Systemic lupus erythematosus-related antiphospholipid syndrome

An analysis of 21 studies (Love and Santoro, 1990) with > 1000 SLE patients found that 34% were positive for LA and 44% for aCL. In a prospective study (Tarr et al., 2007) on 272 SLE patients, 165 (60%) had aPL, 84 of 165 aPL-positive patients fulfilled the criteria for APS over 5-year follow-up, indicating that SLE patients with aPL have a 50% risk of thrombotic complications. The risk of thrombosis in SLE is greater in those with positive LA than with aCL. Neither the titre of anti-DNA antibodies nor the serum complement levels correlate with aPL levels.

Patients with SLE and aPL can develop any of the renal complications seen in primary APS. An isolated thrombotic microangiopathy causing significant renal dysfunction occurs in up to 10% of SLE patients with these antibodies. In addition, glomerular microthrombosis is seen in 10–30% of patients with lupus nephritis, especially in those with severe diffuse proliferative glomerulonephritis: they are associated with more severe proteinuria, worse hypertension, lower glomerular filtration rate (GFR), the development of glomerular sclerosis, and poor renal and patient survival (Tektonidou et al., 2004; Zheng et al., 2009). Lupus nephritis patients with microthrombi are more likely to have aPL (particularly LA) and to have a history of previous thrombotic episodes and/or recurrent fetal loss (Wakai et al., 1991; Daugas et al., 2002). The mechanism underlying the formation of microthrombosis is largely unknown, but is likely to involve complement activation (Cohen et al., 2008). Whether or not patients with glomerular microthrombi should be treated with anticoagulants in the absence of other thrombotic processes remains an open question. Reflecting the common clinical and serological manifestations between SLE and APS it is notable that primary APS may evolve into SLE suggesting that these apparently different diseases are related (Shoenfeld et al., 2009). It is not known to what extent these two conditions share a common genetic background.

Catastrophic antiphospholipid syndrome

CAPS (also known as Asherson syndrome) is an accelerated variant of APS resulting in multiorgan failure. A definitive diagnosis of CAPS requires (a) clinical evidence of involvement of three or more organ systems in a period of less than a week, (b) histopathological evidence of small vessel occlusion in at least one organ system, and (c) laboratory confirmation of the presence of aPL, usually in high

titre (Asherson et al., 2003). Approximately 60% of the catastrophic episodes are preceded by a precipitating event, mainly infection.

The kidney is the organ most commonly affected by CAPS. According to the international CAPS registry data (Cervera et al., 2009), 71% of patients had renal involvement, usually resulting in acute kidney injury, severe hypertension, and laboratory evidence of glomerular damage (proteinuria and haematuria). Renal biopsy revealed the typical frank microangiopathy. Immune complex nephritis was seldom encountered. Renal infarctions were also present in some patients.

CAPS is uncommon but potentially life-threatening with a 50% mortality rate. The presence of SLE is the only identified poor prognostic factor for a higher mortality rate in patients with CAPS. Causes of death include major organ involvement (other than the kidney) and infection (Bucciarelli et al., 2009). A high index of suspicion, early diagnosis, and aggressive therapy are vital for the survival of CAPS patients.

Antiphospholipid antibodies in end-stage renal disease

Studies in ESRD have shown a high prevalence of aPL in patients on haemodialysis (10–30%), with a lower incidence in those on peritoneal dialysis. These antibodies were independent of age, length of time on dialysis, sex, type of dialysis membrane, drugs, and chronic B and C hepatitis (Brunet et al., 1995). Although the mechanisms involved in the genesis of aPL in ESRD are unknown, they appear to be β_2GP1 independent. Possible causes include dialysis membranes, trauma to blood passing through the haemodialysis circuit, and microbial contamination of the dialysate. APL probably increase the risk of thrombosis of haemodialysis vascular access but this has yet to be conclusively demonstrated.

Antiphospholipid antibodies in renal transplantation

Renal transplantation is associated with significant morbidity and mortality in patients with APS, and should be undertaken with caution. The presence of aPL may damage the renal allograft but is also associated with post-transplant mortality and non-renal APS manifestations. In one study (Ducloux et al., 1999) of non-SLE patients, 28% of 178 transplant patients had aPL that were associated with a three- to fourfold increased risk of arterial and venous thrombosis. SLE patients with aPL have a particularly poor outcome following renal transplant from allograft loss or other vascular complications, with up to 60% risk for developing clinical events associated with APS (Stone et al., 1999). However, a study (Forman et al., 2004) of 337 renal transplant recipients found the 18% who were IgG or IgM aCL-positive (even after correction for the effects of anticoagulation) had no greater allograft loss or reduction in GFR over time than did patients who were aCL negative. Around 16% of patients develop *de novo* aPL after transplantation, possibly caused by infection or rejection. These patients have a greater thrombotic risk than those with pre-transplant aPL. Screening for aPL should be considered in any patient experiencing a thromboembolic event following renal transplantation. aPL-positive patients with pre-emptive transplantation (no previous history of dialysis) are also at increased risk, suggesting that aPL that develops during dialysis may be less pathogenic. In addition, HCV-positive renal transplant patients with aCL appear to have a higher risk of thrombotic microangiopathy in the allograft (Baid et al., 1999). Identifying high-risk patients through pre-transplant screening for pro-thrombotic risk, including aPL, may reduce the transplant mortality and morbidity, and risk of allograft failure.

Thrombotic microangiopathy can recur in the allograft and has responded to the complement C5 inhibitor eculizumab, indicating a role for complement in the pathogenesis of this lesion (Hadaya et al., 2011).

Treatment

The optimal treatment of patients with aPL or the APS is a subject of controversy. Many patients with aPL do not experience thrombotic events and do not require anticoagulation or other special therapy. However, non-SLE individuals with high titres of IgG aPL and no previous thrombosis may require long-term primary thromboprophylaxis with low-dose aspirin, especially in the presence of other thrombotic risk factors. Patients with SLE and positive LA or isolated persistent aCL at medium–high titres should receive primary thromboprophylaxis with hydroxychloroquine and low-dose aspirin. The current recommendations for treating APS, either primary or SLE related, include heparin followed by long-term warfarin as long as the abnormal antibody persists. In general, intermediate-intensity treatment with warfarin (international normalized ratio (INR) 2.0–3.0) is sufficiently effective in most patients with a first episode of venous thrombosis who do not have a major risk of bleeding. In patients with arterial events or with recurrent thrombotic events, however, a higher high-intensity treatment (INR 3.0–4.0) or an additional antiplatelet agent may be required (Lim et al., 2006). Direct factor Xa inhibitors, such as rivaroxaban, have entered the clinic for warfarin-intolerant patients, but their efficacy in APS management is unclear.

Thrombotic microangiopathy in APS has been treated with plasma exchange along with anticoagulation. Other manifestations of APS, including vasculopathy, livedo reticularis, and proteinuria, insufficiently controlled by anticoagulation, have also been treated with corticosteroids with or without immune suppressives. Both plasma exchange and rituximab reduce aCL levels and have been reported to be effective but lack firm evidence

In pregnancy, prophylactic therapy with unfractionated heparin or low-molecular-weight heparin with or without aspirin is indicated for APS, particularly in the presence of prior pregnancy loss (Petri and Qazi, 2006). Women with previous renal disease from APS and renal impairment are at high risk of complications during pregnancy and in the puerperium. To improve the outcomes of pregnancies in such women, a closer obstetric surveillance and multidisciplinary clinics, including nephrologists, are essential.

Although high-dose corticosteroids may reduce the titre of aPL, the effect is frequently lost as the drug is tapered. Therefore corticosteroid treatment is not a reasonable long-term approach. The current treatment of CAPS has emerged from study of reports to the international CAPS registry (Cervera and Espinosa, 2012); besides identification and treatment of any precipitating factor, first-line therapies should always include the combination of anticoagulation and corticosteroids plus intravenous immunoglobulin and/or plasma exchange. Although immunosuppression with cyclophosphamide or mycophenolate mofetil would be indicated in the context of an SLE flare, this worsens the prognosis of CAPS.

New therapeutic modalities such as rituximab, defibrotide, and eculizumab may have a role in the treatment of patients with CAPS in the future.

Conclusions

The renal manifestations of APS are heterogeneous and form a continuum between primary APS and SLE. APS manifestations should be considered and looked for in all SLE patients. Over 20% of lupus nephritis patients will have features of APS that need to be considered in planning management and can confer a worse prognosis. Certain manifestations of APS require specific treatment, such as plasma exchange for TMA, and anticoagulation for thromboembolism. Data is scarce on the effects of or indications for immune suppressive therapy in APS. A severe form of APS, CAPS, can involve the kidneys and carries a high mortality risk.

References

Alchi, B., Griffiths, M., and Jayne, D. (2010). What nephrologists need to know about antiphospholipid syndrome. *Nephrol Dial Transplant* 25, 3147–54.

Amigo, M. C. (2006). Kidney disease in antiphospholipid syndrome. *Rheum Dis Clin North Am* 32, 509–22.

Asherson, R. A., Cervera, R., de Groot, P. G., *et al.* (2003). Catastrophic antiphospholipid syndrome: international consensus statement on classification criteria and treatment guidelines. *Lupus* 12, 530–4.

Asherson, R. A., Cervera, R., Shepshelovich, D., *et al.* (2006). Nonthrombotic manifestations of the antiphospholipid syndrome: away from thrombosis? *J Rheumatol*, 33, 1038–44.

Baid, S., Pascual, M., Williams, W. W. Jr., *et al.* (1999). Renal thrombotic microangiopathy associated with anticardiolipin antibodies in hepatitis C-positive renal allograft recipients. *J Am Soc Nephrol*, 10, 146–53.

Biggioggero, M. and Meroni, P. L. (2010). The geoepidemiology of the antiphospholipid antibody syndrome. *Autoimmun Rev*, 9, A299–304.

Brunet, P., Aillaud, M. F., San Marco, M., *et al.* (1995). Antiphospholipids in hemodialysis patients: relationship between lupus anticoagulant and thrombosis. *Kidney Int*, 48, 794–800.

Bucciarelli, S., Espinosa, G., and Cervera, R. (2009). The CAPS Registry: morbidity and mortality of the catastrophic antiphospholipid syndrome. *Lupus*, 18, 905–12.

Cervera, R., Bucciarelli, S., Plasín, M. A., *et al.* (2009). Catastrophic antiphospholipid syndrome (CAPS): descriptive analysis of a series of 280 patients from the 'CAPS Registry.' *J Autoimmun*, 32, 240–5.

Cervera, R. and Espinosa, G. (2012). Update on the catastrophic antiphospholipid syndrome and the 'CAPS Registry.' *Semin Thromb Hemost*, 38, 333–8.

Chaib, A. I., Mellilo, N., Sangle, S. R., *et al.* (2007). Antiphospholipid antibodies and increased bleeding complications following renal biopsy: a single centre study. *Arthritis Rheum*, 56, S740.

Cohen, D., Koopmans, M., Kremer Hovinga, I. C., *et al.* (2008). Potential for glomerular C4d as an indicator of thrombotic microangiopathy in lupus nephritis. *Arthritis Rheum*, 58, 2460–9.

Daugas, E., Nochy, D., Huong, D. L., *et al.* (2002). Antiphospholipid syndrome nephropathy in systemic lupus erythematosus. *J Am Soc Nephrol*, 13, 42–52.

D'Cruz, D. (2009). Renal manifestations of the antiphospholipid syndrome. *Curr Rheumatol Rep*, 11, 52–60.

De Laat, H. B., Derksen, R. H., Urbanus, R. T., *et al.* (2004). beta2-glycoprotein I-dependent lupus anticoagulant highly correlates with thrombosis in the antiphospholipid syndrome. *Blood*, 104, 3598–602.

Ducloux, D., Pellet, E., Fournier, V., *et al.* (1999). Prevalence and clinical significance of antiphospholipid antibodies in renal transplant recipients. *Transplantation*, 67, 90–3.

Fakhouri, F., Noël, L. H., Zuber, J., *et al.* (2003). The expanding spectrum of renal diseases associated with antiphospholipid syndrome. *Am J Kidney Dis*, 41, 1205–11.

Forman, J. P., Lin, J., Pascual, M., *et al.* (2004). Significance of anticardiolipin antibodies on short and long term allograft survival and function following kidney transplantation. *Am J Transplant*, 4, 1786–91.

Galli, M., Luciani, D., Bertolini, G., *et al.* (2003). Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. *Blood*, 101, 1827–32.

Giannakopoulos, B., Passam, F., Rahgozar, S., *et al.* (2007). Current concepts on the pathogenesis of the antiphospholipid syndrome. *Blood*, 109, 422–30.

Griffiths, M. H., Papadaki, L., and Neild, G. H. (2000). The renal pathology of primary antiphospholipid syndrome: a distinctive form of endothelial injury. *QJM*, 93, 457–67.

Hadaya, K., Ferrari-Lacraz, S., Fumeaux, D., *et al.* (2011). Eculizumab in acute recurrence of thrombotic microangiopathy after renal transplantation. *Am J Transplant*, 11, 2523–7.

Hughes, G. R. (1983). Thrombosis, abortion, cerebral disease, and the lupus anticoagulant. *Br Med J*, 287, 1088–9.

Lim, W., Crowther, M. A., and Eikelboom, J. W. (2006). Management of antiphospholipid antibody syndrome: a systematic review. *JAMA*, 295, 1050–7.

Love, P. E. and Santoro, S. A. (1990). Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. *Ann Intern Med*, 112, 682–98.

Mehrani, T. and Petri, M. (2011). IgM anti-ß2 glycoprotein I is protective against lupus nephritis and renal damage in systemic lupus erythematosus. *J Rheumatol*, 38, 450–3.

Meroni, P. L., Borghi, M. O., Raschi, E., *et al.* (2011). Pathogenesis of antiphospholipid syndrome: understanding the antibodies. *Nat Rev Rheumatol*, 7, 330–9.

Miyakis, S., Lockshin, M. D., Atsumi, T., *et al.* (2006). International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost*, 4, 295–306.

Muscal, E., Edwards, R. M., Kearney, D. L., *et al.* (2011). Thrombotic microangiopathic hemolytic anemia with reduction of ADAMTS13 activity: initial manifestation of childhood-onset systemic lupus erythematosus. *Am J Clin Pathol*, 135, 406–16.

Nochy, D., Daugas, E., Droz, D., *et al.* (1999). The intrarenal vascular lesions associated with primary antiphospholipid syndrome. *J Am Soc Nephrol*, 10, 507–18.

Parmar, K., Lefkou, E., Doughty, H., *et al.* (2009). The utility of the Taipan snake venom assay in assessing lupus anticoagulant status in individuals receiving or not receiving an oral vitamin K antagonist. *Blood Coagul Fibrinolysis*, 20, 271–5.

Petri, M. and Qazi, U. (2006). Management of antiphospholipid syndrome in pregnancy. *Rheum Dis Clin North Am* 32, 591–607.

Ruiz-Irastorza, G., Crowther, M., Branch, W., *et al.* (2010). Antiphospholipid syndrome. *Lancet*, 376, 1498–509.

Sangle, S. R., D'Cruz, D. P., Abbs, I. C., *et al.* (2005). Renal artery stenosis in hypertensive patients with antiphospholipid (Hughes) syndrome: outcome following anticoagulation. *Rheumatology (Oxford)*, 44, 372–7.

Sangle, S. R., D'Cruz, D. P., Jan, W., *et al.* (2003). Renal artery stenosis in the antiphospholipid (Hughes) syndrome and hypertension. *Ann Rheum Dis*, 62, 999–1002.

Shen, Y., Chen, X. W., Sun, C. Y., *et al.* (2010). Association between anti-beta2 glycoprotein I antibodies and renal glomerular C4d deposition in lupus nephritis patients with glomerular microthrombosis: a prospective study of 155 cases. *Lupus*, 19, 1195–203.

Shoenfeld, Y., Meroni, P. L., and Toubi, E. (2009). Antiphospholipid syndrome and systemic lupus erythematosus: are they separate entities or just clinical presentations on the same scale? *Curr Opin Rheumatol*, 21, 495–500.

Sinico, R. A., Cavazanna, I., Nuzzo, M., *et al.* (2010). Renal involvement in primary antiphospholipid syndrome: retrospective analysis of 160 patients. *Clin J Am Soc Nephrol*, 5, 1211–17.

Stone, J. H., Amend, W. J., and Criswell, L. A. (1999). Antiphospholipid antibody syndrome in renal transplantation: occurrence of clinical events in 96 consecutive patients with systemic lupus erythematosus. *Am J Kidney Dis*, 34, 1040–7.

Tarr, T., Lakos, G., Bhattoa, H. P., *et al.* (2007). Clinical thrombotic manifestations in SLE patients with and without antiphospholipid antibodies: a 5-year follow-up. *Clin Rev Allergy Immunol*, 32, 131–7.

Tektonidou, M. G., Sotsiou, F., and Moutsopoulos, H. M. (2008). Antiphospholipid syndrome (APS) nephropathy in catastrophic, primary, and systemic lupus erythematosus-related APS. *J Rheumatol*, 35, 1983–8.

Tektonidou, M. G., Sotsiou, F., Nakopoulou, L., *et al.* (2004). Antiphospholipid syndrome nephropathy in patients with systemic lupus erythematosus and antiphospholipid antibodies: prevalence, clinical associations, and long-term outcome. *Arthritis Rheum*, 50, 2569–79.

Wakai, S., Matsumura, O., Koike, T., *et al.* (1991). Anti-cardiolipin antibody and renal microthrombi in lupus nephritis. *Ryumachi*, 31, 481–7.

Willis, R., Harris, E. N., and Pierangeli, S. S. (2012). Pathogenesis of the antiphospholipid syndrome. *Semin Thromb Hemost*, 38, 305–21.

Zheng, H., Chen, Y., Ao, W., *et al.* (2009). Antiphospholipid antibody profiles in lupus nephritis with glomerular microthrombosis: a prospective study of 124 cases. *Arthritis Res Ther*, 11, R93.

CHAPTER 165

The patient with scleroderma: systemic sclerosis

Bernadette Lynch and Aine Burns

Introduction

Scleroderma in its systemic form (systemic sclerosis (SSc)) is a debilitating, chronic, (autoimmune) disease of unknown cause characterized by vasculopathy and progressive fibrosis most notably of the skin (Barnes and Mayes, 2012). The typical clinical features of scleroderma are well described and illustrated in Fig. 165.1. The histological hallmark is excess collagen deposition around capillaries in the skin. Other internal organs including lungs, heart, gastrointestinal tract, as well as kidneys may also be affected and these often determine prognosis. The disease is more common in women usually presenting between the ages of 30 and 60 years. Skin changes usually begin in the fingers (sclerodactyly) and may progress rapidly to involve arms, face, anterior chest wall, lower limbs, and abdomen. Extensive facial telangiectasia may occur and Raynaud's phenomenon almost always accompanies the disease. Microstomia and xerostomia (as part of a more generalized sicca syndrome) are often present together with poor dentition and dysphagia resulting from both dry mouth and oesophageal dysmotility (Dedic et al., 2011). Lung involvement may be in the form of pulmonary fibrosis and/or pulmonary hypertension. Restrictive cardiomyopathy and right heart failure secondary to pulmonary hypertension are well recognized. Small pericardial effusions are frequently identified but larger compromising effusions have also been reported. In addition to the problems with oesophageal dysmotility, gastrointestinal symptoms are common with abdominal discomfort, bloating, and constipation predominating. Poor dentition resulting from microstomia (which makes dental work difficult) and reduced saliva production is frequent. Small bowel bacterial overgrowth, large bowel diverticula (but uncommonly diverticulitis), and malabsorption complete the clinical spectrum.

Terminology and definitions

Scleroderma occurs in two predominant, clinically distinct, forms: limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc) systemic sclerosis. LcSSc is diagnosed when skin sclerosis occurs distal to the wrists (or ankles) or over the face and neck but not extending elsewhere. Such patients often suffer longstanding Raynaud's phenomenon. DcSSc is characterized by truncal as well as acral skin involvement. The typical puffy or hidebound skin changes usually occur within 1 year of onset of Raynaud's phenomenon and progress steadily although the skin involvement may precede the onset of vascular symptoms. Tendon friction rubs are also characteristic of dcSSc. However, the terminology used to describe the extent and location of the skin changes can be confusing and is summarized in Table 165.1.

Although considered a skin condition, extensive vascular abnormalities, in addition to Raynaud's phenomenon, are evident both in the skin and in other affected organs. Historically, both angiography and histopathology of larger blood vessels (including intrarenal vessels) clearly demonstrate narrowing, tapering, and occlusion. Pathologically, similar vascular changes are recognized in the kidney and may be present without either hypertension or abnormal biochemistry (Leinwand et al., 1954; Hannigan et al., 1956; D'Angelo et al., 1969; Cannon et al., 1974).

Renal involvement in scleroderma

For well over a century, renal involvement in scleroderma has been known to occur. In crisis form (referred to as scleroderma renal crisis (SRC)) it is one of the most dramatic and devastating complications of this disease (D'Angelo et al., 1969; Cannon et al., 1974). The first case was described in 1863 (Kaiser, 2009) and later in 1892 Sir William Osler wrote: 'Patients with SSc are…. apt to succumb to pulmonary complaints or to nephritis' (Cunningham et al., 1980). However, Moore and Sheehan who also warned of the dire prognostic significance of its occurrence published the first detailed description of SRC (Moore and Sheehan, 1952). Up until the 1970s most patients suffering SRC died within 3 months to 1 year. Survival even in a tertiary referral centre was reported to be < 20% (Moore and Sheehan, 1952). By the mid 1970s, there were isolated reports of prolonged survival following bilateral nephrectomies and initiation of chronic dialysis (Shapiro et al., 1977). In 1983, Traub et al. described their experiences of a group of 68 patients with progressive systemic sclerosis admitted to hospitals of the University of Pittsburgh Health Center between 1955 and 1981 with SRC as follows:

> The onset of SRC was characterized by four features, namely, onset or aggravation, usually abrupt, of arterial hypertension; appearance of Grade III or IV retinopathy; elevations of peripheral renin activity to at least twice the upper limit of normal; and rapid deterioration of renal function within a period of less than one month. Over 90% of our patients in whom these criteria could be determined had at least three of them present with the onset of SRC. Management of these patients during the first 15 years of this period was uniformly ineffective. Before 1971, no patients lived longer than a year; usual survival ranged from 1 to 3 months. With the advent of renal dialysis and

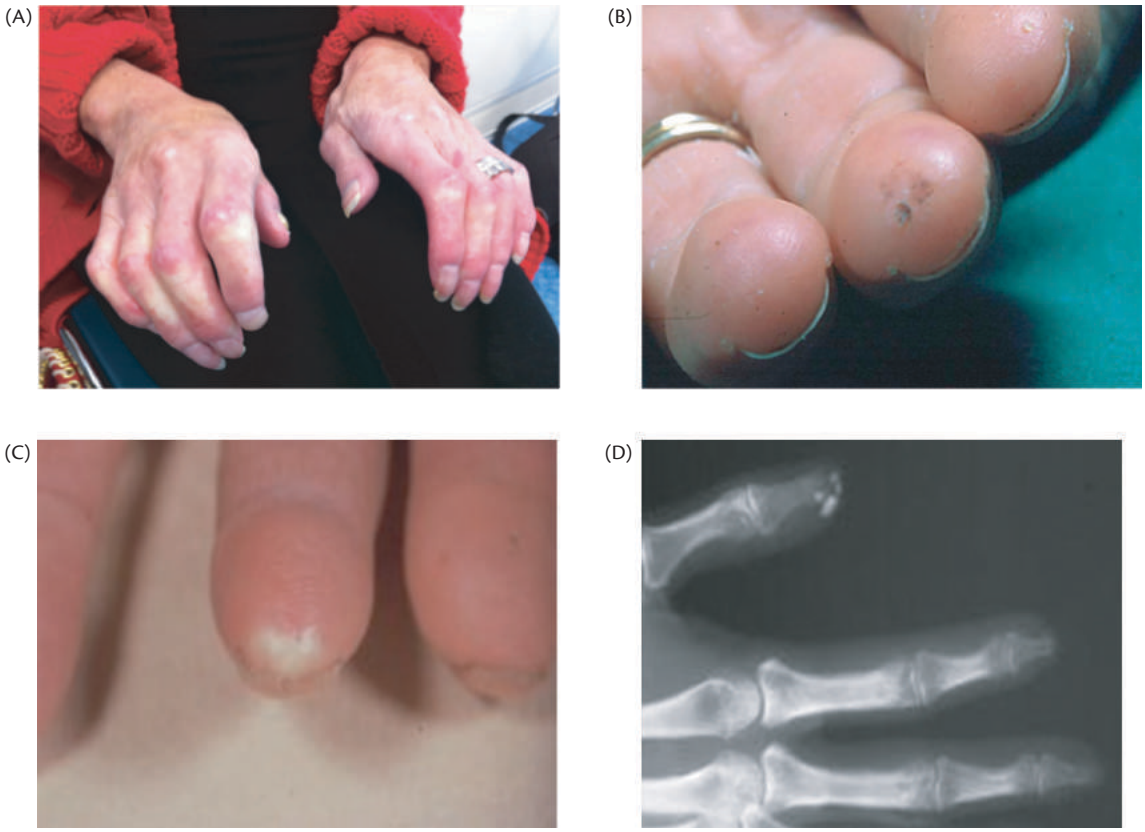

Fig. 165.1 Clinical features of scleroderma showing typical shiny, atrophic skin and Raynaud's of hands (A) with dry ulcers at the tip of middle finger (B) and calcinosis (C) which is illustrated on plain radiograph of the hand at the thumb (D).

> the more effective treatment of severe hypertension, along with the utilization of bilateral nephrectomy in selected anuric patients, some improvement in longevity was achieved (Traub et al., 1983, p. 335).

By the late 1970s, angiotensin-converting enzyme inhibitors (ACEIs) had become available and mortality fell dramatically from 85% at 1 year to 24% (Wasner et al., 1978; Zawada et al., 1981; Steen and Medsger, 2007). This transformed mortality represents one of the landmark advances in the management of SRC. However, the long-term survival after SRC remains poor, especially for the substantial number of patients who require long-term dialysis, whose mortality at 5 years remains between 30% and 60% (Steen and Medsger, 2000; De Marco et al., 2002; Penn et al., 2007; Teixeira et al., 2007).

Table 165.1 Terminology

Term	Definition
Scleroderma	Tightness, thickening and non-pitting induration of skin
Sclerodactyly	Skin changes in the hands and feet listed above distal to the MCP or MTP joints
Proximal scleroderma	Changes listed above proximal to the MCP or MTP joints
Diffuse cutaneous scleroderma (dcSSc)	Truncal and acral skin involvement. Presence of tendon friction rubs. Onset of skin changes (puffy or hidebound) within 1 year of onset of Raynaud's phenomenon – Skin involvement may precede onset of vascular symptoms
Limited cutaneous scleroderma (lcSSc)	Skin sclerosis distal to the wrists (or ankles), over the face and neck. Often longstanding Raynaud's phenomenon

Aetiology and pathogenesis of scleroderma renal crisis

The sequence of events leading to a SRC is not fully understood. Clearly, patients with anti-RNA polymerase III—positive autoantibody (ARA) profiles are over-represented. Steroids (Trang et al., 2012) and ciclosporin (Denton et al., 1994; Radstake et al., 2011) are well recognized to precipitate crises. The detrimental effects of steroids may reflect increased vascular sheer stresses consequent on their mineralocorticoid effects. Drugs like ciclosporin may cause direct vascular endothelial injury and narrowed lumens. Analgesics (non-steroidal anti-inflammatory drugs) perhaps via their anti-prostaglandin effects as well as pain secondary to digital ischaemia and increased sensitivity to cold or temperature changes are all reported as relevant. Kovalchik et al. have demonstrated exaggerated production of renin by SSc patients subjected to remote cold stimuli when compared with control individuals (Kovalchik et al., 1978). Human leucocyte antigen (HLA), angiotensin-converting enzyme, connective-tissue growth factor (CTGF), and endothelin receptor A genotypes have been linked to SSc and SRC but other genetic susceptibility and unrecognized factors may also prevail (Mayer et al., 2002; Fonseca et al., 2006, 2007; Joung et al., 2006; Williams et al., 2009; Radstake et al., 2010; Nguyen et al., 2011).

Table 165.2 Frequency and clinical associations of hallmark systemic sclerosis (SSc) associated auto-antibodies

Autoantibodies	Frequency (%)	Subset Associations	Organ complication associations
Anti-centromere	16–40	lcSSc	Protective for PF & SRC
Anti-topoisomerase I	9–39	dcSS > lcSS	PF; SDV
Anti-RNA polymerase	4–25	dcSSc	SRC
Anti-Th/To	0.2–7	lcSSc	PF; PH
Anti-U3RNP	1–6	dcSSc > lcSSc; SSc overlap with PM/DM	PH
Anti-U11/U12 RNP	1.6–5	dcSSc = lcSSc	PF, gastrointestinal involvement
Anti-PM-Scl	0–7	SSc overlap with PM/DM or RA	PF

dcSSc diffuse cutaneous systemic sclerosis; *lcSSc* limited cutaneous systemic sclerosis; *PH* pulmonary hypertension; *PF* pulmonary fibrosis; *PM/DM* polymyositis/dermatomyositis; *SDV* severe digital vasculopathy; *SRC* scleroderma renal crisis; *RA* Rheumatoid arthritis

The aetiology of SSc is unknown but much evidence supports an autoimmune basis for its development. In recent years, research on SSc has evolved to provide a better understanding of the interdependence of the three major systems involved; namely, the vascular system, the immune system, and the connective tissue. In the earliest stages of skin involvement a large influx of mononuclear cells (mostly activated T lymphocytes) infiltrates the skin and surround blood vessels. Several mediators synthesized by immune cells, including cytokines such as transforming growth factor beta (TGF-β) and platelet-derived growth factor (PDGF), cooperate in inducing the activation of fibroblasts and their differentiation into myofibroblasts. It is believed that this immunological activity leads to the exaggerated production of collagen. Genetic and environmental factors are likely to be relevant but their exact role has yet to be determined (Shi-Wen et al., 2007; Hunzelmann and Brinckmann, 2010; Radstake et al., 2010; Barnes and Mayes, 2012). Hypoxia is increasingly recognized as a decisive factor in modulating the inflammatory process in SSc, activating fibroblasts and changing their phenotype.

A number of autoantibodies that have been identified in scleroderma patients are directed against various specific cellular components and correlate with distinct subsets of the disease (Sato et al., 2001; Codullo et al., 2009; Nhtyanova et al., 2009; Radstake et al., 2011; Barnes and Mayes, 2012). In general, they have a relative high specificity but their sensitivity is only moderate. Table 165.2 summarizes the autoantibodies and their correlation with disease subsets.

ARA provide a strong marker of susceptibility to developing SRC. Thirty per cent of ARA-positive patients in a UK series developed SRC and approximately 60% of cases of SRC in the same cohort were ARA positive. The presence of ARA has also been associated with genetic markers using a candidate gene approach. Polymorphisms in the coding region of the endothelin receptor A gene, in particular, are associated with the presence of ARA in patients with SSc (Fonseca et al., 2006). There are also clear associations between HLA types and ARA as for other SSc specific autoantibodies (Nguyen et al., 2011).

Epidemiology of scleroderma renal crisis

SRC occurs in approximately 5–10% of all SSc cases (2% with lcSSc and 12% with dcSSc). It is more common early on in the progressive phase of dcSSc with the majority of cases occurring within 2 years of disease onset. Penn et al. reported a median duration of SSc at time of SRC of 7.5 months (range 0–200 months) with 66% of patients suffering SRC within 1 year of diagnosis of SSc (Penn et al., 2007). Rarely, it has been reported to occur in those without obvious or even occasionally preceding skin changes (Gore and Brown, 2006). However, SRC was the presenting feature of SSc for 22% of patients in Penn's series and this late diagnosis was associated with a poorer outcome (Penn et al., 2007). It is unknown why only a minority of patients with SSc develop SRC. A second major or multiple, minor, triggers as well as genetic susceptibility are likely, in addition to the SSc. Unrecognized renal abnormalities are common in both incident and prevalent patients including renal vascular abnormalities, non-nephrotic range proteinuria (although nephrotic range proteinuria has been described (Nepal et al., 2008)), subtle excretory impairment, and undiagnosed hypertension. Early angiographic images demonstrated these vascular abnormalities both prior to and following the development of SRC. Some workers have described the renovascular changes coincident with SRC as a 'Raynaud's phenomenon' occurring within the kidney.

Clinical features of scleroderma renal crisis

Clinically, SRC is characterized by the development of severe hypertension (often described as accelerated or malignant) together with acute kidney injury (AKI). Evidence of microangiopathic haemolytic anaemia, oliguria, cardiac failure, and tachyarrhythmias may also be present.

Although the reported incidence of SRC in SSc varies depending on whether limited disease is included or what definitions of SRC are used, the true figure is likely to be circa 5% of patients with dcSSc and 2% of those with lcSSc. Penn et al. using strict definitions of both SRC (Table 165.3) and disease type reported a series of 1997 UK patients presenting between 1990 and 2005 of whom 110 developed SRC: of these 24 (22%) suffered from lcSSc (compared with database prevalence of 65%) while 86 (78%) suffered cdSSc (database 35%) (Penn et al., 2007).

Investigations

The presence of ARA and recent initiation or intensification of steroid therapy (> 15 mg per day) together with rapidly progressing skin scores are recognized as risk factors, others are listed in Box 165.1 (Teixeira et al., 2007; Denton et al., 2009; Nihtyanova et al., 2012; Trang et al., 2012). Anaemia, thrombocytopenia, and new cardiac events may arise as early consequences of the SRC rather than representing true risk factors yet they serve as useful alerts to the possibility of SRC. The prevalence of ARA in patients whose disease is progressing to renal crisis is at least five times as high as the prevalence in patients who do not develop SRC. Conversely, anti-Scl-70 antibodies though present in 70% of SSc patients are under-represented in the SRC population occurring in only 30%.

Table 165.3 Renal Crisis Classification

Definition of SRC
◆ New onset of blood pressure >150/85mm Hg obtained at least twice over a 24-hour period
◆ Documented decrease in the renal function as defined by a decrement of > 30% in the calculated glomerular filtration rate (eGFR)
Corroborative features:
◆ Micro-angiopathic haemolytic anaemia
◆ Hypertensive retinopathy
◆ New-onset of urinary RBCs (other causes having been excluded)
◆ Flash pulmonary oedema
◆ Oligouria or anuria
◆ Typical renal biopsy features (Figure 4)

These findings have facilitated targeted vigilance and early antihypertensive therapy in those most at risk.

A typical patient undergoing a SRC presents with new or worsening (usually severe) hypertension or with its consequences such as headache, blurred vision (with grade 3–4 retinopathy), and hypertensive encephalopathy, including cortical blindness and seizures. Progressive breathlessness and/or palpitations, occasionally chest pain with evidence of biochemical renal impairment, low urinary sodium, and high urine specific gravity may be evident or oligo-anuria if the AKI is advanced at the time of presentation.

The hypertensive crisis usually, although not exclusively, occurs in patients with known SSc during a phase of rapidly worsening skin disease. Extreme vasoconstriction of the peripheral circulation with marked (marble-like) coldness of the extremities has been observed. Box 165.2 outlines the prior treatments of the patients undergoing SRC in Penn et al.'s cohort of British SRC patients. In the most severely affected individuals, systemic vascular resistances (SVRs) of up to five times normal (> 4000 dyn·s/cm^5 normal in health = 1000) have been measured. The left ventricle (LV) struggles to maintain stroke volume in the face of such elevated peripheral resistance. Consequently, impressive tachycardias and tachyarrhythmias develop as physiological responses to maintain cardiac output that may also be impaired by underlying restrictive cardiomyopathy and myocardial ischaemia secondary to cardiac vasculopathy. Pulmonary arterial wedge pressures rise and pulmonary oedema may ensue as the LV fails to cope. Thirty-three per cent of Penn's 45 patients with SRC where echocardiography was available shortly after presentation with SRC had ejection fractions < 55%. Electrocardiographs (ECGs) recorded during a SRC would be expected to confirm tachycardia and to demonstrate left ventricular strain. Features of right heart strain occur as a late sign or if pulmonary hypertension may also be present. Proteinuria and haematuria (implying severe cortical ischaemia) may be present on dipstick urine analysis. Some patients undergoing a renal crisis have concurrent, overt, evidence of the underlying profound vasculopathy with painful digital gangrene often involving multiple fingers developing in the very early stages of the SRC (Fig. 165.2). Indeed, Smith et al. have recently suggested that worsening capillaroscopy findings may predict future severe organ involvement (Smith et al., 2012).

Box 165.1 Risk factors for developing SRC

- Diffuse skin disease
- Rapidly progressive skin disease (< 4 years since scleroderma onset)
- High skin score
- Large joint contractures
- New cardiac events
- Steroid usage (exceeding 15 mg per day)
- Ciclosporin treatment
- Presence of anti-RNA-polymerase III auto-antibodies (ARA)
- Anaemia and thrombocytopenia
- Endothelin receptor A and angiotensin-converting enzyme (*ACE*) gene polymorphism.

Box 165.2 Prior treatment in a cohort of SRC patients (Penn et al., 2007)

64 patients had full medication history available prior to SRC:

- 59% treated with steroids within 1 month prior to SRC
- 39% taking disease-modifying drugs at time of SRC (5 anti-thymocyte globulin, 4 ciclosporin, 4 azathioprine, 3 interferon-α, 2 D-penicillamine, 2 cyclophosphamide, 2 methotrexate, 1 anti-transforming factor-β antibody, 1 mycophenolate mofetil, 1 danazole)
- 18/68 were prescribed ACEI at time of crisis
- 2/68 were prescribed ARB at time of crisis.

Clinically, SRC should be suspected when AKI develops in SSc patients. Nevertheless, AKI occurring in SSc patients is not always due to SRC. Renal artery stenosis, hypovolemia, crescentic glomerulonephritis (GN), and other renal diseases may also occur in SSc patients (Endo et al., 1994; Morris et al., 1994). These disorders may result in a similar clinical picture. In SSc patients, most of the encountered crescentic GN are antineutrophil cytoplasmic antibody (ANCA) associated. These are pauci-immune on immunofluorescence studies, associated with anti-myeloperoxidase antibodies, and usually triggered by penicillamine. A small proportion of patients also suffer from SLE overlap and a variety of glomerular pathologies have been found in them.

The cardinal feature of SRC is sustained hypertension. Mean blood pressure (BP) readings at presentation with SRC were 193/114 mmHg in the cohort of 72 patients described by Penn et al. (2004). Evidence of AKI with rising levels of urea and creatinine, hyperkalaemia, and acidosis may be present. Penn published median creatinine levels of 200 mmol/L at presentation amongst 76 patients with full data. In the same series, evidence of intravascular haemolysis or microangiopathic haemolytic anaemia (MAHA) was found in about half (reduced platelet counts 50%, red cell fragments 52%). Reduced serum haptoglobin levels, red cell fragments and schistocytes on blood film together with massively elevated lactate dehydrogenase levels and importantly normal clotting provide further evidence of MAHA.

Chest radiograph may reveal evidence of pulmonary oedema or background pulmonary fibrosis. An enlarged cardiac silhouette might result from a pericardial effusion. Echocardiography is useful to exclude clinically significant effusions, assess pulmonary pressures, and to measure ejection fractions and identify any

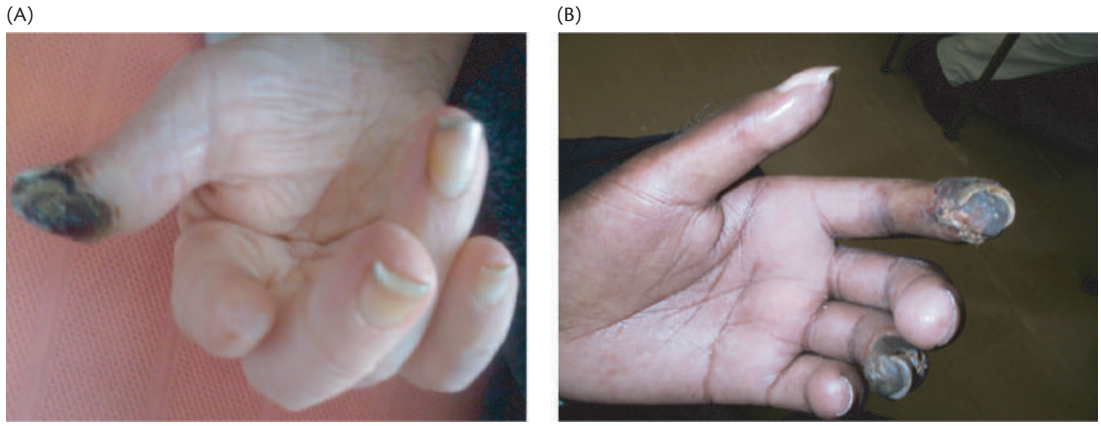

Fig. 165.2 Gangrenous thumb (A) and index and ring finger with telangiectasia of the palm and thumb (B).

coexisting valvular abnormalities. Most patients presenting with SRC have non-significant pericardial effusions although haemodynamically significant effusions can occasionally cloud the picture.

Troponin and pro-brain natriuretic peptide may be useful indicators of myocardial ischaemia and failure respectively but the latter is certainly useful where there is coexisting pulmonary hypertension (Black and Coghlan, 2006).

In new SSc patients, cold pressor testing, performed by immersing a patient's hands in cold water, demonstrates increased and prolonged sensitivity with thermal maps indicating very cold peripheries and slow warming compared with controls. Nail-fold capillaries become sparse elongated and tortuous in SSc patients (Fig. 165.3). These latter tests can be helpful in those patients who do not have a known or obvious diagnosis of SSc at presentation or in whom the skin changes are minimal or absent.

Histology of the kidney

The renal pathological findings in SRC are indistinguishable from any other cause of accelerated hypertension (Batal et al., 2009). Renal biopsies are considered to be helpful (to exclude other pathologies and assess prognosis) but the timing of renal biopsy clearly needs to be delayed until the patient's BP is well controlled, the clinical condition of the patient is stable, and platelet counts have recovered. Vessels show profound intimal proliferation that may occlude the vessel lumen completely and fibrinoid necrosis

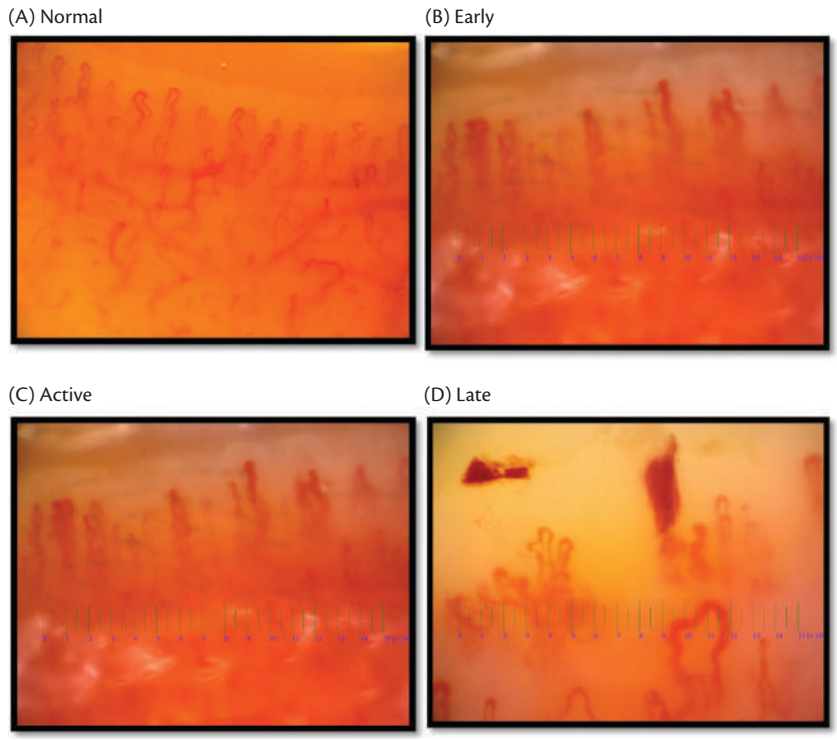

Fig. 165.3 Nail-fold capillaroscopy.

(A) Normal. (B) Early changes with dilated vessels and minimal drop-out. (C) More active pattern with dilation of vessels. (D) Later pattern with architectural derangement with drop-out.

Images courtesy of Kevin Howell, Department of Rheumatology, Royal Free Hampstead, London, UK.

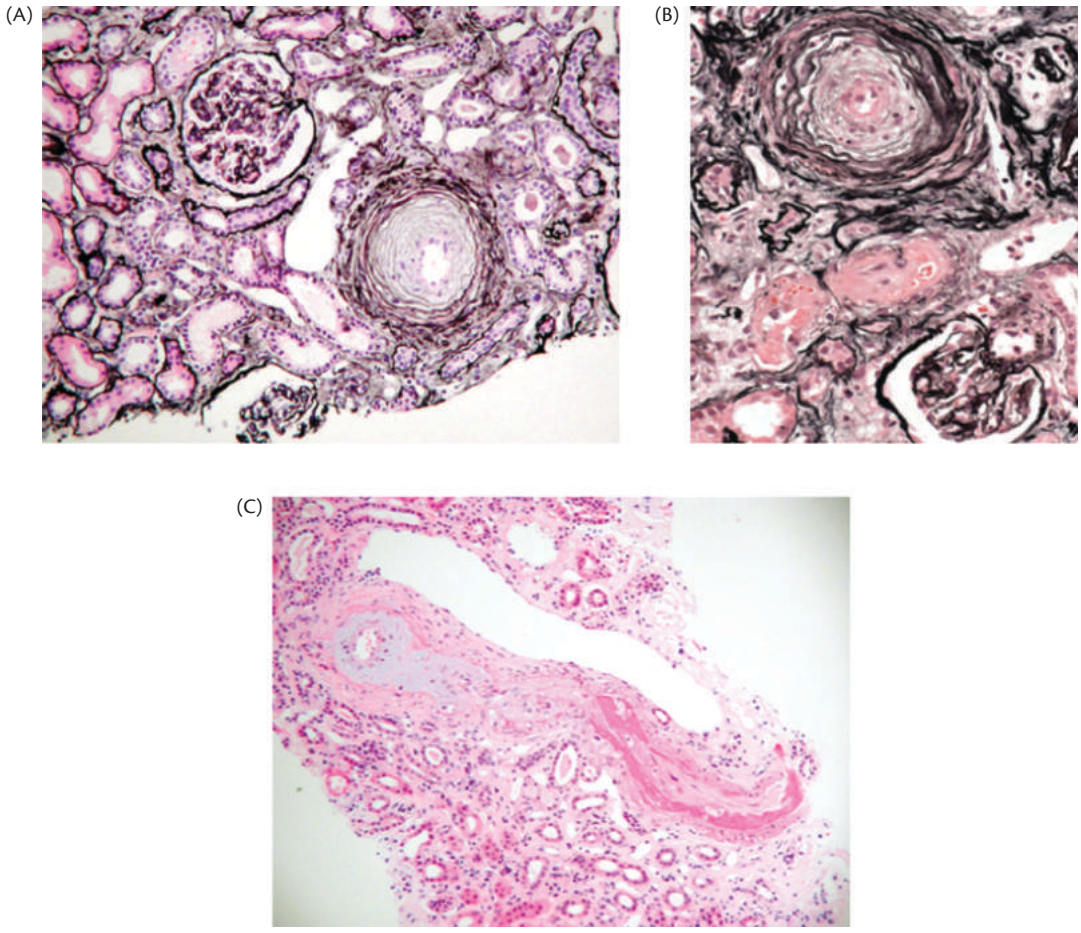

Fig. 165.4 Histological appearances of a typical renal biopsy taken following a SRC. Severe acute small vessel vasculopathy. (A and B) Show shrunken collapsed glomeruli with wrinkling of basement membranes particularly (B) (silver stain). Interlobular arteries show virtual occlusion by loose concentric intimal thickening (onion skinning). (C) Figure is a longitudinal section through a very abnormal, almost completely occluded vessel (haematoxylin and eosin stain).
Images courtesy of Professor A. J. Howie, Department of Histopathology, Royal Free Hampstead, London, UK.

may be present in vessel walls. Glomeruli become collapsed with wrinkling of basement membrane (Fig. 165.4). The prognostic value of measurements of renal scarring does not follow the patterns seen in other renal diseases (Penn et al., 2007).

Treatment and outcome

Acute management of SRC involves general supportive care with thoughtful BP control. Prompt BP control is essential if hypertensive encephalopathy or cardiac de-compensation dictate it. Otherwise, moderate, steady reduction in BP (10% reduction in systolic BP per day) is likely to optimize chances of renal recovery. The use of an ACEI or angiotensin-II receptor blocker (ARB) in the early stages is now standard and there is evidence that continuation of these agents even if the patient becomes dialysis dependent improves the chances of recovering sufficient renal function and becoming dialysis independent (Zawada et al., 1981; Steen and Medsger, 2007). Intravenous vasodilators especially prostaglandin inhibitors are effective in the short term and the latter may have the added advantage of discouraging platelet/vascular endothelial activation and are beneficial for digital ischaemia and pulmonary disease (Medsger et al., 2001; Rubin et al., 2002; Korn et al., 2004; Dhaun et al., 2009). Intravenous prostaglandin inhibitors, in particular, can be titrated effectively in very sick patients to reduce SVR and increase stroke volume with consequent slowing of heart rate and improvement of cardiac index and cardiac failure. This type of goal-directed management can be facilitated using oesophageal Doppler or Swann–Ganz monitors in an intensive therapy unit or high-dependency setting. In severely tachycardia patients, beta blockers may be contraindicated and should be used with extreme caution as the increased heart rate maintains cardiac output in the setting of such high SVR and reduced stroke volume.

The short-term outcomes of SRC have improved greatly over the last half-century (Steen and Medsger, 2000, 2007; Black and Medsger, 2004; Penn et al., 2007; Denton et al., 2009; Dhaul et al., 2009). Increased awareness of this complication with regular BP measurements especially in susceptible groups allows earlier diagnosis with improved outcomes. There is still uncertainty as to whether pre-emptive ACEI is beneficial (Hudson et al., 2010). Recent series report crisis survival rates of 70–80% although overall in Penn et al.'s series 63% of patients required dialysis at presentation and 33% of survivors were still on dialysis at 5 years. However, 23% of the total recovered sufficient renal function to be able to discontinue dialysis although the median time to becoming dialysis independent was 11 months (range 1–34 months). Annual estimated glomerular filtration rate (eGFR) of those not dialysed or who were able to discontinue dialysis generally showed ongoing improvement for at least 3 years. Survival whether on dialysis or

Table 165.4 Short- and long-term Outcome of SRC (Penn 2007)

Short term outcome of SRC

- 36% did not require dialysis during SRC (3 subsequently needed dialysis 7, 8 and 10 years later)
- 23% recovered sufficient function to discontinue dialysis (2 required dialysis again)
- 42% dialysis without recovery (including 3 who refused dialysis and died)
- 19 patients died on dialysis
- 3 renal transplant (one peri-operative death)
- Higher blood pressure at presentation was associated with a better outcome
- Low LVEF at time of SRC was not associated with requiring permanent dialysis
- Older patients were no more likely to require dialysis but were less likely to recover renal function if dialysis was required
- No correlation between presentation eGFR and renal outcome
- No correlation between MAHA and outcome
- No correlation between steroid use and renal outcome
- Insufficient data to determine whether prior treatment with ACEi/ARB had a significant influence on outcome

Long-term Survival following SRC

- 82% - 1 year
- 74% - 2 years
- 71% - 3 years
- 59% - 5 years
- 47% - 10 years
- Most common in the dialysis recovery group
- The dialysis and recovery group had the best prognosis
- No correlation between age at time of SRC and death
- Prognosis was worse in males (17% ten-year survival)

not is poor in this patient group with men and those remaining on dialysis faring worst (Table 165.4). Interestingly, many authors have reported spontaneous improvement in skin scores following a SRC (Korn et al., 2004; Rubin et al., 2007; Shand et al., 2007).

For dialysis dependent patients, renal transplantation is an option but careful consideration needs to be given to the timing of transplantation as renal recovery can occur up to 2 years following a SRC. Similarly, a suitable immunosuppressive agent needs to be selected bearing in mind that ciclosporin is known to precipitate SRC. Furthermore, coexisting cardiac and pulmonary disease may dictate suitability for listing (Pham et al., 2005). Although in general renal transplantation offers superior survival in SRC patients, graft survival is reduced compared to the general renal transplant population and recurrence of scleroderma may play a role in this poor post-renal transplant outcome. In one series, two of 10 patients had histologic features suspicious for SRC recurrence (Batal et al., 2007). Both early and late recurrence has been reported (Chung et al., 2005; Pham et al., 2005).

Otherwise therapy for SSc is organ and pathogenesis targeted. In a review, using MEDLINE and the Cochrane Registry, of open trials and controlled trials only, for treatment of SSc from 1999 to April 2005, Zandman-Goddard et al. identified methotrexate, ciclosporin, tacrolimus, relaxin, low-dose penicillamine, and intravenous immunoglobulin (IVIg) as beneficial in improving the skin tightness in SSc (Zandman-Goddard et al., 2005). Calcium channel blockers, the angiotensin II receptor type 1 antagonist losartan, prazocin, the prostacyclin analogue iloprost, *N*-acetylcysteine, and the dual endothelin-receptor antagonist bosentan were concluded to be potentially beneficial for Raynaud's phenomenon whilst they noted epoprostenol and bosentan are approved for therapy of pulmonary arterial hypertension. Cyclophosphamide pulse therapy was found to be effective in suppressing active alveolitis. They concluded that stem cell and lung transplantation were viable options for carefully selected patients. They reaffirmed that SRC should be effectively managed by aggressively controlling hypertension with ACEIs and that patients should continue taking ACEIs even after beginning renal replacement therapy in hope of discontinuing dialysis. Antithymocyte globulin and mycophenolate mofetil appeared safe in SSc (Zandman-Goddard et al., 2005). Clearly, more prospective trials are needed to help clarify optimal therapy for SSc and its complications.

Conclusion

Despite recent improvements in overall survival in SSc and advances in organ-based therapies, SRC remains an important complication of the disease. An estimated 10–15% of dcSSc patients may develop SRC. Patients with SRC may recover renal function up to 3 years after the crisis, most often within 12–18 months. Early, aggressive therapy with ACEIs and other antihypertensive agents is essential to reduce the morbidity and mortality.

References

Barnes, J. and Mayes, M. D. (2012). Epidemiology of systemic sclerosis: incidence, prevalence, survival, risk factors, malignancy, and environmental triggers. *Curr Opin Rheumatol*, 24(2), 165–70.

Batal, I., Bastacky, S., and Randhawa, P. (2007). The outcome of kidney transplantation in patients with scleroderma. *Am J Transplant,* 7(2), 363.

Batal, I., Domsic, R. T., Shafer, A., *et al.* (2009). Renal biopsy findings predicting outcome in scleroderma renal crisis. *Hum Pathol*, 40(3), 332–40.

Black, C. M. and Medsger, T. A. (2004). Dr Leroy's contribution to the understanding of systemic sclerosis. *Clin Exp Rheumatol*, 22(3 Suppl 33), S3–4.

Black, C. M. and Coghlan, J. G. (2006). Role of N-terminal brain natriuretic peptide (N-TproBNP) in scleroderma-associated pulmonary arterial hypertension. *Eur Heart J*, 27(12), 1485–94.

Cannon, P. J., Hassar, M., Case, D. B., *et al.* (1974). The relationship of hypertension and renal failure in scleroderma (progressive systemic sclerosis) to structural and functional abnormalities of the renal cortical circulation. *Medicine (Baltimore)*, 53(1), 1–46.

Cheung, W. Y., Gibson, I. W., Rush, D., *et al.* (2005). Late recurrence of scleroderma renal crisis in a renal transplant recipient despite angiotensin II blockade. *Am J Kidney Dis,* 45(5), 930–4.

Codullo, V., Cavazzana, I., Bonino, C., *et al.* (2009). Serologic profile and mortality rates of scleroderma renal crisis in Italy. *J Rheumatol,* 36(7), 1464–9.

Cunningham, P. H., Andrews, B. S., and Davis, J. S., 4th. (1980). Scleroderma: developments from Osler to the present. *South Med J*, 73(6), 770–4.

D'Angelo, W. A., Fries, J. F., Masi, A. T., *et al.* (1969). Pathologic observations in systemic sclerosis (scleroderma). A study of fifty-eight autopsy cases and fifty-eight matched controls. *Am J Med*, 46(3), 428–40.

Dedic, A., Pasalic, A., Hodzic, M., *et al.* (2011). Scleroderma—from the aspect of dental medicine. *Med Arch*, 65(6), 368–70.

Denton, C. P., Lapadula, G., Mouthon, L., *et al.* (2009). Renal complications and scleroderma renal crisis. *Rheumatology (Oxford)*, 48 Suppl 3, iii32–5.

Denton, C. P., Sweny, P., Abdulla, A., *et al.* (1994). Acute renal failure occurring in scleroderma treated with cyclosporin A: a report of three cases. *Br J Rheumatol,* 33(1), 90–2.

DeMarco, P. J., Weisman, M. H., Seibold, J. R., *et al.* (2002). Predictors and outcomes of scleroderma renal crisis: the high-dose versus low-dose D-penicillamine in early diffuse systemic sclerosis trial. *Arthritis Rheum*, 46(11), 2983–9.

Dhaun, N., MacIntyre, I. M., Bellamy, C. O., *et al.* (2009). Endothelin receptor antagonism and renin inhibition as treatment options for scleroderma kidney. *Am J Kidney Dis*, 54(4), 726–31.

Endo, H., Hosono, T., and Kondo, H. (1994). Antineutrophil cytoplasmic autoantibodies in 6 patients with renal failure and systemic sclerosis. *J Rheumatol*, 21(5), 864–70.

Fonseca, C., Renzoni, E., Sestini, P., *et al.* (2006). Endothelin axis polymorphisms in patients with scleroderma. *Arthritis Rheum*, 54(9), 3034–42.

Fonseca, C., Lindahl, G. E., Ponticos, M., *et al.* (2007). A polymorphism in the CTGF promoter region associated with systemic sclerosis. *N Engl J Med*, 357(12), 1210–20.

Gore, J. E. and Brown, A. N. (2006). Scleroderma renal crisis sine scleroderma in pregnancy: a case report. *Clin Exp Rheumatol*, 24(1), 87–8.

Hannigan, C. A., Hannigan, M. H., Scott, E. L. (1956). Scleroderma of the kidneys. *Am J Med*, 20(5), 793–7.

Horn, H. C., Ottosen, P., and Junker, P. (2001). Renal crisis in asclerodermic scleroderma--lupus overlap syndrome. *Lupus*, 10(12), 886–8.

Hudson, M., Baron, M., Lo, E., *et al.* (2010). An international, web-based, prospective cohort study to determine whether the use of ACE inhibitors prior to the onset of scleroderma renal crisis is associated with worse outcomes-methodology and preliminary results. *Int J Rheumatol,* 2010, 347402.

Hunzelmann, N. and Brinckmann, J. (2010). What are the new milestones in the pathogenesis of systemic sclerosis? *Ann Rheum Dis*, 69 Suppl 1, i52–6.

Joung, C. I., Park, Y. W., Kim, S. K., *et al.* (2006). Angiotensin-converting enzyme gene insertion/deletion polymorphism in Korean patients with systemic sclerosis. *J Korean Med Sci*, 21(2), 329–32.

Kaiser, H. (2009). [A dermatologist and a rheumatologist define a syndrome. George Thibierge (1856–1926), Raymond J. Weissenbach (1885–1963)]. *Z Rheumatol*, 68(7), 594–8, 600–1.

Korn, J. H., Mayes, M., Matucci Cerinic, M., *et al.* (2004). Digital ulcers in systemic sclerosis: prevention by treatment with bosentan, an oral endothelin receptor antagonist. *Arthritis Rheum*, 50(12), 3985–93.

Kovalchik, M. T., Guggenheim, S. J., Silverman, M. H., *et al.* (1978). The kidney in progressive systemic sclerosis: a prospective study. *Ann Intern Med,* 89(6), 881–7.

Locke, I. C., Worrall, J. G., Leaker, B., *et al.* (1997). Autoantibodies to myeloperoxidase in systemic sclerosis. *J Rheumatol*, 24(1), 86–9.

Lwinwand, I., Duryee, A. W., and Richter, M. N. (1954). Scleroderma; based on a study of over 150 cases. *Ann Intern Med,* 41(5), 1003–41.

Mayer, N. J., Forsyth, A., Kantachuvesiri, S., *et al.* (2002). Association of the D allele of the angiotensin I converting enzyme polymorphism with malignant vascular injury. *Mol Pathol*, 55(1), 29–33.

Medsger, T. A., Jr., Morganti, A., Kramer, F., *et al.* (2011). Bosentan treatment of digital ulcers related to systemic sclerosis: results from the RAPIDS-2 randomised, double-blind, placebo-controlled trial. *Ann Rheum Dis,* 70(1), 32–8.

Moore, H. C. and Sheehan, H. L. (1952). The kidney of scleroderma. *Lancet*, 1(6698), 68–70.

Morris, K., Connolly, J. O., O'Donnell, P. J., *et al.* (1994). Malignant hypertension and renal failure: scleroderma renal crisis or renal artery stenosis? *Nephrol Dial Transplant*, 9(10), 1489–91.

Nepal, M., Mainali, R., Schworer, C. M., *et al.* (2008). Nephrotic range proteinuria: rare manifestation of scleroderma renal crisis. *Ann Clin Lab Sci*, 38(2), 163–7.

Nguyen, B., Mayes, M. D., Arnett, F. C., *et al.* (2011). HLA-DRB1*0407 and *1304 are risk factors for scleroderma renal crisis. *Arthritis Rheum*, 63(2), 530–4.

Nihtyanova, S. I., Parker, J. C., Black, C. M., *et al.* (2009). A longitudinal study of anti-RNA polymerase III antibody levels in systemic sclerosis. *Rheumatology (Oxford)*, 48(10), 1218–21.

Penn, H., Howie, A. J., Kingdon, E. J., *et al.* (2007). Scleroderma renal crisis: patient characteristics and long-term outcomes. *QJM*, 100(8), 485–94.

Pham, P. T., Pham, P. C., Danovitch, G. M., *et al.* (2005). Predictors and risk factors for recurrent scleroderma renal crisis in the kidney allograft: case report and review of the literature. *Am J Transplant*, 5(10), 2565–9.

Radstake, T. R., Gorlova, O., Rueda, B., *et al.* (2010). Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. *Nat Genet*, 42(5), 426–9.

Radstake, T. R., Gorlova, O., Rueda, B., *et al.* (2011). Clinical and serological hallmarks of systemic sclerosis overlap syndromes. *J Rheumatol,* 38(11), 2406–9.

Rubin, L. J., Badesch, D. B., Barst, R. J., *et al.* (2002). Bosentan therapy for pulmonary arterial hypertension. [Erratum in *N Engl J Med*, 2002, 346(16), 1258.] *N Engl J Med,* 346(12), 896–903.

Rubin, L. J., Black, C. M., Denton, C. P., *et al.* (2007). Clinical trials and basic research: defining mechanisms and improving treatment in connective tissue disease. *Arthritis Res Ther*, 9 Suppl 2, S10.

Sato, H., Lagan, A. L., Alexopoulou, C., *et al.* (2004). The TNF-863A allele strongly associates with anticentromere antibody positivity in scleroderma. *Arthritis Rheum,* 50(2), 558–64.

Shand, L., Lunt, M., Nihtyanova, S., *et al.* (2007). Relationship between change in skin score and disease outcome in diffuse cutaneous systemic sclerosis: application of a latent linear trajectory model. *Arthritis Rheum*, 56(7), 2422–31.

Shapiro, C. B., Lerner, N. E., Ackad, A. S., *et al.* (1977). Malignant hypertension and uremia in scleroderma: efficacy of nephrectomy and hemodialysis. *Clin Nephrol*, 8(1), 321–3.

Shi-Wen, X., Renzoni, E. A., Kennedy, L., *et al.* (2007). Endogenous endothelin-1 signaling contributes to type I collagen and CCN2 overexpression in fibrotic fibroblasts. *Matrix Biol*, 26(8), 625–32.

Smith, V., Decuman, S., Sulli, A., *et al.* (2012). Do worsening scleroderma capillaroscopic patterns predict future severe organ involvement? a pilot study. *Ann Rheum Dis*, 71(10), 1636–9.

Steen, V. D. and Medsger, T. A. (2007). Changes in causes of death in systemic sclerosis, 1972–2002. *Ann Rheum Dis*, 66(7), 940–4.

Steen, V. D. and Medsger, T. A. Jr. (2000). Long-term outcomes of scleroderma renal crisis. *Ann Intern Med,* 133(8), 600–3.

Teixeira, L., Mouthon, L., Mahr, A., *et al.* (2008). Mortality and risk factors of scleroderma renal crisis: a French retrospective study of 50 patients. *Ann Rheum Dis*, 67(1), 110–16.

Teixeira, L., Mahr, A., Berezné, A., *et al.* (2007). Scleroderma renal crisis, still a life-threatening complication. *Ann N Y Acad Sci*, 1108:249–58.

Trang, G., Steele, R., Baron, M., *et al.* (2012). Corticosteroids and the risk of scleroderma renal crisis: a systematic review. *Rheumatol Int,* 32(3), 645–53.

Traub, Y. M., Shapiro, A. P., Rodnan, G. P., *et al.* (1983). Hypertension and renal failure (scleroderma renal crisis) in progressive systemic sclerosis. Review of a 25-year experience with 68 cases. *Medicine (Baltimore)*, 62(6), 335–52.

Walker, J. G., Ahern, M. J., Smith, M. D., *et al.* (2003). Scleroderma renal crisis: poor outcome despite aggressive antihypertensive treatment. *Intern Med J*, 33(5–6), 216–20

Walker, K. M., Pope J., participating members of the Scleroderma Clinical Trials Consortium (SCTC); Canadian Scleroderma Research Group (CSRG) (2012). Treatment of systemic sclerosis complications: what to use when first line treatments fail—a consensus of systemic sclerosis experts. *Semin Arthritis Rheum,* 42(1), 42–55.

Wasner, C., Cooke, C. R., and Fries, J. F. (1978). Successful medical treatment of scleroderma renal crisis. *N Engl J Med,* 299(16), 873–5.

Williams, M. H., Handler, C. E., Akram, R., *et al.* (2009). Angiotensin-converting enzyme gene does not contribute to genetic susceptibility to systemic sclerosis in European Caucasians. *J Rheumatol*, 36(2), 337–40.

Zawada, E. T., Jr., Clements, P. J., Furst, D. A., *et al.* (1981). Clinical course of patients with scleroderma renal crisis treated with captopril. *Nephron*, 27(2), 74–8.

Zandman-Goddard, G., Tweezer-Zaks, N., and Shoenfeld, Y. (2005). New therapeutic strategies for systemic sclerosis—a critical analysis of the literature. *Clin Dev Immunol*, 12(3), 165–73.

CHAPTER 166

The patient with rheumatoid arthritis, mixed connective tissue disease, Sjögren syndrome, or polymyositis

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Rheumatoid arthritis

Prevalence and patterns of disease

Renal dysfunction is relatively common in patients with rheumatoid arthritis (RA). It is estimated that 13–18% have a glomerular filtration rate (GFR) of ≤ 60 mL/min (Daoussis et al., 2009; Anders and Vielhauer, 2011), 5–35% haematuria, and 3–7% proteinuria (Karstila et al., 2007).

There have been substantial changes in the pattern and frequency of kidney disease in RA. In the 1950s to 1980s, renal failure was an important contributor to the cause of death in 4–27% of cases (Mutru et al., 1976) in patients with RA. Renal amyloid was found in 1.5–9% of cases (Cobb et al., 1953; Rasker and Cosh, 1981; Prior et al., 1984; Laakso et al., 1986). More recent studies however show a significant reduction in the proportion of deaths in RA that are attributable to renal failure (Wolfe et al., 1994; Kroot et al., 2000). Sokka et al. (2008) reviewed 84 published cohorts and found that renal disease contributed to 5.8% of deaths. With more aggressive treatment of RA with disease-modifying drugs, there does seem to be a lessening of the incidence and severity of renal disease.

Autopsy studies in the 1940s showed a proliferative glomerulonephritis in between 13% (Fingerman and Andrus, 1943) and 63% (Baggenstoss and Rosenberg, 1943) of cases. However, infection in these patients may have caused the glomerulonephritis. Subsequently, autopsy studies have differed in the pattern of renal disease seen (Table 166.1). This may be due to selection bias and referral patterns. Thus some studies have shown a high incidence of glomerulonephritis and renal vasculitis (Ramirez et al., 1981; Boers et al., 1987) with little in the way of renal papillary necrosis. In contrast, in the study of Nanra (1975), 30% of patients had a renal papillary necrosis, and no mention was made of either glomerulonephritis or amyloidosis. Varying treatment regimens may account for these differences.

Renal biopsies

Although initial renal biopsy studies in patients with RA and minimal urinary abnormalities showed an almost complete absence of glomerulonephritis, later studies investigated those with significant renal disease and provided good evidence of glomerulonephritis (Table 166.2). No study has compared the prevalence of glomerulonephritis in patients with RA with that in healthy controls. Ideally such a study should be done to establish beyond all argument that glomerulonephritis may develop as a consequence of RA. This is important for two reasons: first, the development of proteinuria and renal impairment in a patient with RA never indicates inevitable renal amyloid (nowadays rarely does), with its inexorable progression to renal failure, and second, because some types of renal disease, such as vasculitis, are amenable to treatment with steroids and cyclophosphamide (see below).

Amyloidosis

There are two aspects of relevance to rheumatic diseases: where primary amyloidosis mimics arthritis, and secondary amyloidosis occurring in patients with RA. (See Chapter 152.)

Rheumatic symptoms in amyloidosis

Rheumatic symptoms occur in the following contexts:

1. Haemodialysis arthropathy, in which an increased plasma β_2-microglobulin concentration results in deposition of fibrils, producing the characteristic clinical picture of an entrapment neuropathy (especially carpal tunnel syndrome), bone cysts, and destructive arthropathy with a predilection for the shoulder and wrist.
2. Amyloidosis that involves articular structures and mimics other rheumatic complaints.

In amyloidosis associated with a monoclonal gammopathy, joint symptoms occur frequently. Here a clinical picture of symmetrical small-joint involvement with subcutaneous nodules can lead to the erroneous diagnosis of RA. Neuropathy (10%) and carpal tunnel syndrome (20%) also occur. Purpura is common and when accompanied by factor X deficiency (due to the increased affinity of amyloid light chain (AL) for factor X), severe haemorrhage may occur. Helpful distinguishing features are that in myeloma stiffness is short-lived, the joints are rarely very tender, erosions are uncommon, and there is often excessive soft tissue swelling for the degree

of inflammation. Also, despite the presence of nodules, the serum does not contain immunoglobulin (Ig)-M rheumatoid factor.

Secondary amyloidosis

An in-depth discussion of secondary amyloidosis is given in Chapter 152. Fortunately, there is evidence for a decline in the prevalence of amyloid in Western Europe. Immonen et al. (2011) showed the incidence of rheumatic disease-associated amyloidosis registered onto the Finnish Registry for Kidney Diseases from 2002 onwards was half of that found in the 1990s. The reason for this is likely to be greater awareness of the harmful effect of inflammation and the acute-phase response, and more aggressive treatment to suppress this.

Table 166.1 Autopsy studies in rheumatoid arthritis

Study	Country	No. of patients	Amyloid (%)	Renal failure (%)
Missen and Taylor, 1956	UK	47	17	
Mutru et al., 1976;	Finland	41	17	27
Ramirez et al., 1981	USA	76	8	9
Boers et al., 1987;	Holland	132	11	23
Suzuki et al., 1994	Japan	81	21	9.9
Koivuniemi et al., 2009	Finland	513		4

Glomerulonephritis in RA

Proliferative glomerulonephritis

A proliferative glomerulonephritis is rare in RA. In the autopsy study of Boers et al. (1987), 5 out of 132 cases had a proliferative glomerulonephritis (diffuse in one and focal in four). However, recent biopsy studies in patients with RA have reported little in the way of proliferative glomerulonephritis.

Mesangial proliferative glomerulonephritis

Recently, several studies in patients with RA and microscopic haematuria have reported a mild mesangial proliferative glomerulonephritis, mostly without immune deposits. Sellars et al. (1983) described a mesangial proliferative glomerulonephritis in 13 of 30 patients. The biopsies of nine were examined by immunofluorescent microscopy, and showed mesangial IgA deposits in two. Helin et al. (1986) reported mild mesangial glomerulonephritis in 11 of

Table 166.2 Renal biopsy studies in rheumatoid arthritis

Study	No. of patients	Clinical status	Normal	Mesangial proliferative glomerulonephritis	Membranous glomerulonephritis	Amyloid	Tubulointerstitial nephritis	Other
Pollak et al., 1962	41	Normal, proteinuria	21	0	0	4	0	16
Brun et al., 1965	32	Normal, proteinuria, renal impairment	11	0	1	4	9	7
Salomon et al., 1974	18	Normal, proteinuria, microscopic haematuria	11	7	0	0	0	0
Orjavik et al., 1981	14	Proteinuria, nephrotic syndrome	0	5	0	7	0	2
Sellars et al., 1983	30	Proteinuria, microscopic haematuria, nephrotic syndrome	0	13	9	1	4	3
Hordon et al., 1984	21	Microscopic haematuria	1	15	1	0	1	3
Helin et al., 1986	39	Proteinuria, nephrotic syndrome, renal impairment	3	11	9	16	0	0
Korpela et al. 1990	74	Not given	7	23	13	20	3	8
Adu et al., 1993	90	Proteinuria, nephrotic syndrome, renal impairment	0	10	18	13	14	35
Nakano et al., 1998	158	Proteinuria, nephrotic syndrome haematuria, renal impairment	20	54	49	30		
Galesic et al., 2009	15	Signs of nephropathy	15	3	1	5	0	8

19 patients with RA and haematuria or proteinuria; 3 of the 11 had glomerular mesangial IgA deposits and a further seven had deposits of IgM, IgG, and C3. Details of these and other studies (Salomon et al., 1974; Orjavik et al., 1981; Hordon et al., 1984) are summarized in Table 166.3. The majority of these patients were taking, or had recently been taking, gold or D-penicillamine, suggesting that their mild mesangial proliferative glomerulonephritis was drug related.

Mesangial IgA glomerulonephritis

(IgA nephropathy is described in Chapter 65). In the studies of Helin et al. (1986), Korpela et al. (1990), and Sellars et al. (1983), several patients were described with a mesangial proliferative glomerulonephritis with mesangial IgA deposits. In most patients, mesangial IgA nephropathy is idiopathic, but it has also been reported in patients with cirrhosis of the liver, dermatitis herpetiformis, mycosis fungoides, arthritis after *Yersinia* infection, and seronegative spondyloarthritis. We reported four patients with RA, microscopic haematuria, and proteinuria in whom renal biopsy showed a mesangial IgA glomerulonephritis (Beaman et al., 1987). Three of these patients had never received gold or D-penicillamine, and in the one patient who had received gold this had been discontinued at least 10 years before presentation. These data provide circumstantial evidence of an association between RA and mesangial IgA nephropathy, although the pathogenetic mechanisms are unclear. It may be relevant that raised serum levels of IgA, and also of IgA rheumatoid factors, are found in some patients with RA. There are no long-term studies of renal function in patients with RA and mesangial IgA glomerulonephritis.

Membranous nephropathy

The most common cause of membranous nephropathy (see Chapter 60) in patients with RA is gold or D-penicillamine therapy, although this is now uncommon as these agents are only rarely used. However, there are now many reports of such patients who had never been on gold or D-penicillamine, and the numbers make it unlikely that this association is coincidental. Honkanen et al. (1987) reported on four patients with RA and membranous nephropathy. Only one of their four patients had received gold and that was 16 years before the renal biopsy. Adu et al. (1993) reported six patients with RA and a membranous nephropathy, none of whom had been treated with D-penicillamine, although two had received gold that was discontinued 17 and 13 years before renal biopsy. There was no evidence of systemic lupus erythematosus (SLE) in the patients in one study (Adu et al., 1993) or in those reviewed by Honkanen et al. (1987), which can cause membranous nephropathy.

Necrotizing and/or crescentic glomerulonephritis

This is usually seen in the context of rheumatoid vasculitis and can be associated with the production of antineutrophil cytoplasmic antibodies (ANCAs), some directed against myeloperoxidase. Crescentic nephritis is described in Chapter 70 and systemic vasculitis in Chapters 157–160.

Table 166.3 Commonly used DMARDs and their renal complications

DMARD	Metabolism	Side effect profile	Renal toxicity	Recommendations
Methotrexate	7-hydroxy-methotrexate renally excreted	Hepatoxicity, bone marrow suppression Rare: pneumonitis	Accumulates in renal failure	Monitor GFR especially when on NSAIDs concurrently Reduce dose in renal failure Avoid if GFR < 30mL/min
Sulphasalazine	Metabolized in colon to 5-aminosalicylic acid and sulphapyridine	Gastrointestinal upset, leucopenia, hepatotoxicity, and orange discoloration of urine	Rare Reports of proteinuria (Helliwell, 1995) and renal failure with chronic interstitial nephritis (Dwarakanath et al., 1992)	Monitor GFR
Hydroxychloroquine	Hepatic metabolism, renally excreted	Rare Macular toxicity in cumulative doses	None known	None
Leflunomide	Renally excreted	Hypertension, reduction of serum phosphate and urate levels (Perez-Ruiz and Nolla, 2003)	Lack of clinical experience	Not recommended in renal impairment
Azathioprine	Metabolites renally excreted	Hepatoxicity, bone marrow suppression. Increased toxicity seen in the minority who have reduced TPMT (thiopurine methyltransferase) levels secondary to a genetic polymorphism.	Rare Reports of interstitial nephritis and renal failure mimicking Goodpasture syndrome (Meys et al., 1992; Stetter et al., 1994)	Reduce dose in severe renal impairment
Cyclophosphamide	Renally excreted	Bone marrow suppression	Haemorrhagic cystitis; mesna with adequate hydration can reduce risk Risk of bladder cancer increases with cumulative dosing	Reduce dose in renal failure. Monitor patients with non-glomerular haematuria (Talar-Williams et al., 1996)

Tubulointerstitial nephritis in association with arthritis

Renal biopsy studies in patients with RA and juvenile chronic arthritis have reported that up to 28% of patients with renal abnormalities have tubulointerstitial nephritis (TIN) (Brun et al., 1965) (see Chapters 83 and 93). This is often attributed to the renal toxicity of analgesic agents, including non-steroidal anti-inflammatory drugs (NSAIDs). It is, however, probable that the arthritic disorders may, in themselves, be associated with the development of TIN.

Juvenile idiopathic arthritis

Renal failure accounts for 38% of deaths in patients with juvenile idiopathic arthritis (JIA). The clinical spectrum of renal involvement in JIA has been reviewed by Anttila (1972). Proteinuria is found in 3–12%, and microscopic haematuria in 3–8% of these patients. Nephrotic-range proteinuria is commonly due to renal amyloid, found in 1.2–6.7% of patients, whilst haematuria and proteinuria may be due to amyloid or gold treatment.

In one study, 47 of 638 patients (7.4%) with JIA had renal disease (Anttila and Laaksonen, 1969). In a study of renal histology (57 renal biopsy, three autopsy) in a relatively unselected group of patients with JIA, renal amyloid was detected in two and an interstitial nephritis in eight patients (Anttila, 1972). There are unexplained geographical variations in the prevalence of amyloidosis in patients with JIA. In the United Kingdom this is 7.4% (Schnitzer and Ansell, 1977), in America 1.8%, and in Poland 10.6% (Filipowicz-Sosnowska et al., 1978).

Renal amyloidosis with end-stage renal failure accounted for 33–50% of deaths in JIA (Schnitzer and Ansell, 1977). Immonen et al. (2008) found the 10-year survival of those with JIA and amyloidosis to be 75%. Ansell et al. demonstrated improved survival with chlorambucil using historical controls for such patients (Ansell et al., 1971). Subsequent uncontrolled studies have confirmed this. In the study of David et al. (1993), 80% of patients treated with chlorambucil were alive at 10 years as compared with 25% of untreated patients. However, clearly there is a need for studies into more modern, safer, alternative therapies.

Antirheumatic drug-induced renal disease

Disease-modifying antirheumatic drugs (DMARDs) play a central role in the treatment of RA. Patients are treated aggressively to control pain, reduce inflammation, and prevent joint erosion and consequent loss of function.

Gold and D-penicillamine nephropathy

Despite intense investigation, the mode of action of neither of these drugs is known. The incidence of toxicity is up to 70%, and withdrawal of the drugs as a result of these adverse reactions occurs in around 64% of patients (Maetzel et al., 2000). With the introduction of newer agents, the use of these particular DMARDs is declining.

In general, gold and D-penicillamine produce very similar problems, and the following discussion applies to both drugs. The side effects can be due to a direct toxic effect of the drug, including anorexia and rashes, or have an immunological basis occurring after 3 months and maximally around 6 months, including renal toxicity, glomerulonephritis, and thrombocytopenia. Drug-induced autoimmune disorders such as SLE (D-penicillamine) occur less frequently (Emery and Panayi, 1989). Unlike gold, the prevalence of side effects increases with the dose of D-penicillamine (Williams et al., 1983).

Along with a poor sulphoxidation ability, human leucocyte antigen (HLA)-DR3 is a significant risk factor for renal toxicity. The latter confers a relative risk of 14.0–32.0 for gold-induced nephropathy and 3.2–10.0 for D-penicillamine-induced nephropathy (Klouda et al., 1979).

The most frequent presenting feature is proteinuria, occurring in approximately 10% of patients receiving gold and up to 30% of patients taking D-penicillamine. This progresses to the nephrotic syndrome in 30% and 16%, respectively. Haematuria is relatively rare (seen more frequently with D-penicillamine) and requires the exclusion of other causes. Renal function is not usually impaired.

Histopathology

Membranous glomerulonephritis

Eighty per cent of patients who present with D-penicillamine- or gold-induced proteinuria will have a membranous glomerulonephritis. Epimembranous 'spikes' and a mild increase in mesangial cells are usually seen, and the diagnosis can be confirmed with immunofluorescence/immunoperoxidase microscopy, which shows granular subepithelial deposits of predominantly IgG. On electron microscopy, electron-dense deposits are seen.

Mesangial glomerulonephritis

Mesangial glomerulonephritis is found in patients with RA irrespective of therapy. The prevalence is probably increased after treatment with DMARDs, and for gold at least there is an increased association between this histological appearance and haematuria.

Immunofluorescence may reveal either granular deposits of immunoglobulin (predominantly IgG) and complement, or may be negative, particularly in the case of D-penicillamine-induced disease.

Minimal-change nephropathy

This can occur in association with the use of DMARDs (Lee et al., 1965). Electron microscopy shows fusion of epithelial-cell foot processes.

Tubulointerstitial disease

This is found in up to 10% of patients in association with mild, low-molecular-weight proteinuria and enzymuria. The outlook is good, with rapid resolution on withdrawal of the drug

Crescentic glomerulonephritis

D-penicillamine may lead to the development of a rapidly progressive glomerulonephritis (Almirall et al., 1993), the clinical picture of Goodpasture syndrome (Gibson et al., 1976), and also a renal vasculitis (Falck et al., 1979).

Management

Renal toxicity due to D-penicillamine and gold has a benign long-term outcome following withdrawal of the drug (Hall et al., 1988). The management of renal toxicity occurring *de novo* on therapy is now determined by practical issues. These include the response of the patient, the amount of proteinuria, and the presence of any deterioration in renal function. In general, a declining albumin, proteinuria > 2 g/24 hours, or a reduced GFR are considered indications for stopping treatment. No specific immunosuppression is required, although supportive measures are given as indicated. Renal biopsy should be confined to those who have deteriorating renal function, or who fail to improve after withdrawal

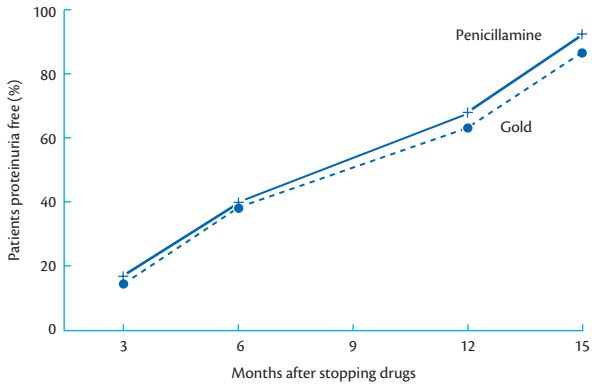

Fig. 166.1 Percentage of patients free of proteinuria after discontinuing gold or penicillamine (Hall et al., 1987, 1988).

of the drug. Regular monitoring of proteinuria and GFR are mandatory. Haematuria, unless it resolves when the drug is stopped, requires investigation in the usual manner.

Long-term outcome

After cessation of the drug, proteinuria peaks at around a month then gradually disappears; the majority of patients will have clear urine by 1 year and almost all will achieve this by 2 years (Fig. 166.1) (Hall et al., 1988). If membranous nephropathy is present, the renal histology reverts towards normal although iterative renal biopsies are rarely needed. Re-challenge with the same drug at the same dose usually leads to a recurrence of the renal problem, although a lower dose may be tolerated.

Ciclosporin nephrotoxicity

The renal toxicity of ciclosporin in RA is well documented (Cohen and Appel, 1992); in these patients, ciclosporin should be started at a dose of 2.5 mg/kg per day and not exceeding 5 mg/kg per day, with a reduction of ciclosporin dosage if creatinine increases to 130% of baseline (Cush, 1999). Indeed, so sensitive is the increase in creatinine in patients with RA that other measures of renal function are used only to confirm changes. This arises from an (initially reversible) afferent arteriolar vasoconstriction. Ciclosporin has been successfully combined with other agents, including methotrexate, the concern here being that the reduction in GFR affects renal excretion of methotrexate. The nephrotoxicity of ciclosporin is increased by NSAIDs (Berg et al., 1989) (see Chapter 362).

Ciclosporin can lead also to chronic, irreversible renal failure with interstitial fibrosis (see Chapter 87), which is more common with doses in excess of 5 mg/kg, in patients with pre-existing renal impairment, in elderly patients, and in patients treated for > 6 months. Renal function should be carefully monitored in patients on ciclosporin therapy.

Other DMARDs

A summary of the effects of commonly used DMARDs are shown in Table 166.3.

Biological therapy

Anti-tumour necrosis factor therapy

Adalimumab (human monoclonal antibody), infliximab (chimeric monoclonal antibody), and etanercept (fusion protein) are the three anti-tumour necrosis factor (TNF) drugs commonly used in rheumatic diseases. They rarely cause renal disease (Smolen and Emery, 2011). Cases of necrotizing crescentic glomerulonephritis have been described but they are mostly associated with the production of ANCA (Fournier et al., 2009; Kaneko et al., 2010). There are no reports as yet of renal complications with the newer anti-TNF agents: golimumab (human monoclonal antibody) and certolizumab pegol (pegylated human monoclonal antibody).

Non-tumour necrosis factor biological therapy

No cases of renal toxicity have been reported with abatacept (T-cell co-stimulatory modulator) and tocilizumab (human monoclonal antibody against IL-6). Rituximab, a chimeric monoclonal antibody against CD20 found on B cells, has made a significant contribution to the management of RA. There is a slightly higher risk of infection and of an infusion reaction; more commonly with the first infusion (Buch et al., 2011), but there are no reports of renal toxicity.

Analgesic nephropathy

This is covered in Chapter 87.

Overlap syndromes

Some patients with a connective tissue disorder do not easily fit into the accepted definitions of a single disease, but present with features overlapping several connective tissue diseases. Although, initially seeming a heterogeneous group of diseases, the overlap syndromes can be subdivided clinically and serologically. The two most common overlap syndromes are described here.

Mixed connective tissue disease

Sharp et al. (1972) reported on 25 patients in whom there was an overlap of the clinical features of SLE, systemic sclerosis, and polymyositis. Sera from all these patients contained antibodies to an RNAase-sensitive, extractable nuclear antigen subsequently shown to be ribonucleoprotein (RNP). They termed this disorder mixed connective tissue disease (MCTD). Subsequent studies have included patients with features at onset similar to RA and have emphasized the asynchronous development of overlapping clinical features of different connective tissues in this syndrome (Wolfe et al., 1994). Most patients are female and present with Raynaud's phenomenon and swollen hands. There may be features of SLE, systemic sclerosis, or polymyositis.

Renal disease occurs in 10–26% of adult MCTD patients (Kitridou et al., 1986) and 27–44% patients with juvenile MCTD (Ito et al., 2006). The clinical presentation usually takes the form of asymptomatic proteinuria or haematuria, often with mild renal impairment. In the study of Kitridou et al. (1986), 75% of patients with renal disease developed a nephrotic syndrome, 42% hypertension, and 17% progressed to chronic renal failure.

Histological changes

Kitridou et al. (1986) reported on the renal histological changes in 12 patients with MCTD and reviewed previous reports in 64 patients. Membranous nephropathy and mesangial proliferative glomerulonephritis were the most common histological cases, found in 34% and 30% of cases, respectively. A focal or diffuse proliferative glomerulonephritis was found in 17%, a mixed lesion with membranous nephropathy in 5%, and in 7% renal histology was normal. Seventeen per cent of patients had evidence of

vascular sclerosis, a feature also emphasized in the autopsy study of Sawai et al. (1994). Immunofluorescence microscopy of glomeruli in patients with MCTD has shown immunoglobulin and complement deposits; on electron microscopy, dense deposits are found consistent with an immune complex-mediated glomerulonephritis (Kitridou et al., 1986). Similar glomerular findings have been reported in children, but in addition widespread intimal proliferation was seen (Singsen et al., 1980). There are rare reports of myeloperoxidase-ANCA-associated microscopic polyangiitis with crescentic glomerulonephritis (Kitaura et al., 2006), and a collapsing glomerulopathy (Rifkin et al., 2011).

Treatment

Treatment of renal failure in MCTD is with steroids initially in high doses, subsequently tapering to a maintenance low dose over weeks. In the study of Kitridou et al. (1986), treatment with high-dose steroids of patients with a nephrotic syndrome was associated with a significant reduction of proteinuria in 62% of episodes. Whether patients resistant to steroids would benefit from the addition of immunosuppressant drugs is not known, but in practice they are added. Fourteen per cent of patients with MCTD and renal involvement reviewed by Kitridou et al. (1986) developed chronic renal failure. The long-term overall mortality of MCTD has varied from 7% over a mean follow up of 7 years (Sharp et al., 1972) to 20–30% over a period of 3–25 years after onset of disease (Nimelstein et al., 1980; Burdt et al., 1999).

Jo-1 (tRNA synthetase) syndrome

Muscle specific antibodies were thought to be a specific finding of myositis but recent studies have led to the identification of separate disease entities (Hengstman et al., 2004). Jo-1 (tRNA synthetase) syndrome is characterized by myositis, interstitial lung disease, Raynaud's phenomenon, arthralgia/arthritis, and 'mechanic's hands'. It is associated with anti-aminoacyl-tRNA synthetase (anti-ARS) antibodies; of which the anti-Jo-1 antibody is the most commonly detected. Mesangial proliferative glomerulonephritis can occur in tRNA synthetase syndrome but is rare (Frost et al., 1993).

Sjögren syndrome

Sjögren syndrome is a progressive autoimmune disease primarily of the exocrine glands, with a prevalence of 0.5% (Voulgarelis et al., 2008). The disease is classified into primary Sjögren syndrome with the disease occurring independently, and secondary Sjögren syndrome in association with other autoimmune disorders such as RA or SLE. Anti-Ro (SS-A) and anti-La (SS-B) antibodies are strongly associated.

The disease is characterized by periepithelial lymphocytic infiltration of the exocrine glands, with associated lymphocytic invasion beyond the exocrine glands into epithelial tissues, and extra-epithelial immune complex deposition. Keratoconjunctivitis sicca and xerostomia are the hallmark symptoms. Extraglandular manifestations include fatigue, arthritis/arthralgia, Raynaud's phenomenon, renal involvement presenting either as interstitial nephritis or glomerulonephritis as described below, and less commonly vasculitis affecting the small to medium-sized vessels. In addition, Kassan et al. (1978) found the relative risk of lymphoma was increased 43.8-fold for those with Sjögren syndrome, but subsequent studies suggest a more modest figure with a lifetime risk of 5–10% (Voulgarelis et al., 2008).

Treatment is usually symptomatic. Hydroxychloroquine, with or without low-dose prednisolone, is sometimes used for mild disease, and stronger immunosuppression, with azathioprine or cyclophosphamide, for example, in severe disease. Rituximab has been shown to be of benefit in reducing associated fatigue and oral dryness (Dass et al., 2008; Meijer et al., 2010) and larger studies (TRACTISS and TEARS) are undergoing.

Renal disease in Sjögren syndrome

Overt renal disease in Sjögren syndrome is less common than latent disease with a prevalence of 3–67% (Kaufman et al., 2008; Lin et al., 2010). The most usual pathology is an interstitial nephritis with glomerular disease less frequently reported, the latter occurring as a late event. Kaufman et al. (2008) identified in the literature 180 case reports of renal involvement in Sjögren syndrome. They described seven case series and 57 individual case reports. Eighty-nine patients underwent a renal biopsy revealing interstitial nephritis in 49 cases, glomerulonephritis in 33, and both in seven (Kaufman et al., 2008). Another case series examined 24 renal biopsies carried out over 40 years from a cohort of 7276 patients with Sjögren syndrome. Seventy-one per cent had signs of acute or chronic TIN as the primary lesion, with chronic TIN the most common presentation (65%). Two patients had cryoglobulinaemic glomerulonephritis and two had focal segmental glomerulosclerosis (Maripuri et al., 2009). Ren et al. (2008) described 130 patients in Shanghai with primary Sjögren syndrome and renal involvement. Ninety-one patients had evidence of tubular disorders, and 10 with glomerulonephritis. Lin et al. (2010) published the clinical characteristics of 573 patients with primary Sjögren syndrome, and 33.5% were found to have renal involvement: 22% had proteinuria, 16.7% had renal tubular acidosis, 9.4% renal stones, and 7.2% had renal dysfunction. Sixty-four underwent a renal biopsy: 21 had interstitial nephritis, 18 with interstitial nephritis and glomerular disease, and 23 with glomerular disease alone.

Interstitial nephritis

Clinical features

Lymphocytic infiltration of the renal tubules can lead to interstitial nephritis. The disease can be subclinical, or can present as mild proteinuria or distal renal tubular acidosis with hyposthenuria, hypokalaemia. or hyperchloraemia. Petrovaara et al. (1999) demonstrated 33% of 54 patients with primary Sjögren's syndrome had an inadequate urine acidification capacity through an abnormal ammonium chloride loading test. Distal renal tubular acidosis is usually only discovered through biochemical abnormalities, but significant disease can lead to renal stone formation, nephrocalcinosis, renal impairment, and osteomalacia. Rarely, patients present with hypokalaemic paralysis and cardiac arrest (Kaufman et al., 2008). Proximal renal tubular acidosis is less common with or without Fanconi syndrome (Kaufman et al., 2008; Ren et al., 2008; Maripuri et al., 2009).

Histology

Renal biopsy in interstitial nephritis reveals diffuse or focal plasma cell and lymphocyte infiltration in a pattern akin to that found surrounding the salivary gland in primary Sjögren syndrome, with CD4 T cells predominating (Matsumura et al., 1995). There can be variable tubular atrophy and fibrosis, and immunofluorescence shows few immune deposits (Ren et al., 2008).

Management

In most cases steroids, with or without hydroxychloroquine, have shown to improve outcome (Kaufman et al., 2008; Maripuri et al., 2009). Few patients have received prednisolone and intravenous cyclophosphamide with variable outcome (Goules et al., 2000; Maripuri et al., 2009). Rituximab has been used in the context of TIN to treat sicca symptoms after which renal function has been maintained (Maripuri et al., 2009).

Glomerulonephritis

The most common glomerular lesions seen are that of membranoproliferative glomerulonephritis, membranous glomerulonephritis, and mesangial proliferative glomerulonephritis. Of the 41 cases of glomerular disease described by Lin et al. (2010), 21 had mesangial proliferative glomerulonephritis, 10 membranous glomerulonephritis, four diffuse proliferative glomerulonephritis, and four focal proliferative glomerulonephritis. Goules et al. (2000) described nine patients out of their 471 patient cohort with primary Sjögren syndrome who developed glomerular disease; five patients had membranoproliferative glomerulonephritis and four mesangial glomerulonephritis. There are rare reports of minimal change disease, focal segmental glomerulosclerosis, crescentic glomerulonephritis, and IgA nephropathy (Dussol et al., 1994; Maripuri et al., 2009; Chen et al., 2010; Yang et al., 2011).

The pathogenesis of the glomerular disease remains poorly understood but a key role is played by the deposition of immune complexes, formed by cryoprecipitable IgMκ rheumatoid factor along with polyclonal IgG and -A. Reflecting small vessel involvement, palpable purpura, low C4, and monoclonal cryoglobulins are strong predictors of its development (Skopouli, 2001).

There is a lack of robust evidence in the treatment of glomerulonephritis in the context of primary Sjögren syndrome. Corticosteroids, with the optional addition of cyclophosphamide, have been shown to be beneficial, although there are case reports of the use of plasma exchange and Rituximab (Maripuri et al., 2009).

Interstitial cystitis

Interstitial cystitis or 'chronic painful bladder syndrome' is a rare complication of Sjögren syndrome. Patients present with bladder and/or pelvic pain with irritable voiding symptoms with an absence of organisms on urine culture. In severe cases, an obstructive uropathy can occur (Shibata et al., 2004). Bladder wall biopsy reveals intense inflammation in mucosa and submucosa with lymphoid and mast cells. Antibodies to the muscarinic M3 receptor found on detrusor smooth muscle cells play a role in its pathogenesis (van de Merwe, 2007). Treatment experience is limited but ciclosporin can be effective (Emmungil et al., 2012).

Polymyositis and dermatomyositis

Inflammatory disease of skeletal muscle may be idiopathic or secondary to a wide variety of disorders. Primary adult idiopathic polymyositis usually presents with insidious proximal muscle weakness and muscle pain, with some patients suffering a preceding febrile illness, Raynaud's phenomenon, or arthralgia. Dermatomyositis also involves characteristic skin changes. In both, rarely does severe muscle weakness develop abruptly, associated with myoglobinuria and diagnosed by raised creatinine kinase.

Acute kidney injury has been reported infrequently in patients with polymyositis and dermatomyositis, and this has been attributed to rhabdomyolysis and myoglobinuria (Kim et al., 2005). The clinical evolution of the acute kidney injury is similar to other causes of non-traumatic rhabdomyolysis.

Glomerulonephritis

There are occasional reports of a mesangial proliferative glomerulonephritis with mesangial deposits of immunoglobulin and complement in patients with polymyositis (Takizawa et al., 2007). Membranous glomerulonephritis has been associated with dermatomyositis (Takizawa et al., 2007). Crescentic glomerulonephritis is rarely reported (Chiu et al., 2008). Treatment with high-dose corticosteroids and azathioprine is of benefit in patients with polymyositis and dermatomyositis. Case reports of the use of rituximab in refractory cases are encouraging (Mann et al., 2010).

Rheumatological complications of renal disease, dialysis, and transplant

Kidney failure and kidney transplantation are often complicated by a variety of rheumatological disorders.

Renal osteodystrophy

Patients with chronic kidney disease may develop bone diseases including secondary hyperparathyroidism, osteomalacia and β_2-microglobulin amyloidosis. These are described in detail in Chapter 122.

Osteoporosis

Osteopenia is common after renal transplantation, with transplant recipients having a fracture rate four times higher than the general population (Palmer et al., 2007). Contributory factors include hyperparathyroidism, pre-transplant bone disease, and the effects of corticosteroids and immunosuppression. Corticosteroids inhibit bone formation, promote bone resorption, promote calciuria, and inhibit intestinal calcium absorption. Ciclosporin and tacrolimus may also increase bone turnover and resorption and decrease gonadal steroid synthesis. Genetic factors may also be implicated, with vitamin D receptor polymorphisms playing a role in bone loss.

In the first year post transplant, bone density reduces by 6–15% (Epstein et al., 1995; Arlen and Adachi, 1999), and subsequent years see a loss of 1–2% (Grotz et al., 1995). Prior to renal transplantation, patients should be assessed with dual energy X-ray absorptiometry scanning prior to transplantation, and again at year 1 and 2. Glucocorticoid use should be minimized, and calcium and vitamin D therapy as appropriate should be commenced. Bisphosphonates, given post transplant, may reduce bone loss but should be avoided in women of childbearing age because of the risk of fetal bone disease. The impact of new, much higher potency bisphosphonates is awaited, and calcitonin looks to be promising (Palmer et al., 2007).

Osteonecrosis

This is a well-recognized cause of bone pain after renal transplantation and is largely due to steroids. With the use of steroid-sparing regimens post renal transplant the incidence has fallen from 20% to 4%. The main symptoms are of severe joint pain. The best imaging technique for diagnosis is magnetic resonance imaging (MRI). Management is mainly symptomatic (rest, analgesics). Surgical intervention (arthroplasty, core decompression) may need to be

considered. Hemi-surface replacement of the femoral head is a newer technique best considered before significant joint destruction and collapse occurs.

Acute bone pain syndrome and reflex sympathetic dystrophy

Acute bone pain syndrome or calcineurin inhibitor-induced pain syndrome (CIPS) affects between 19% and 27% of patients after a renal transplant. It can be difficult to differentiate this from reflex sympathetic dystrophy which may also occur after a renal transplant and which has been linked to treatment with calcineurin inhibitors. CIPS has been reported with both ciclosporin and tacrolimus. MRI shows bone marrow oedema in the affected areas. The presentation is of symmetrical lower limb pain in the first few months after renal transplantation. The best way of treating CPS is unknown. It seems reasonable to reduce the calcineurin inhibitor to the lower end of the therapeutic blood levels or consider a switch to other immunosuppressants. However, both approaches may increase the risk of rejection. Some reports suggest that calcium channel inhibitors especially nifedipine and nitrendipine may reduce the bone pain (Grotz et al., 2001).

Acute hot joint

Septic arthritis should always be considered in the case of an acute hot joint. Infectious complications are common in transplant recipients, and opportunistic organisms such as mycobacteria and fungi, as well as common pathogens, should be considered. Impaired host defences secondary to underlying renal disease or immunosuppression, and pre-existing joint damage are all contributors to the risk of septic arthritis post-transplant (Vincenti et al., 1982). A particular problem occurs in those patients requiring joint replacement post transplantation. Sepsis usually occurs in a single joint and most commonly within 18 months of transplant. Gram-negative joint infections have been associated with concurrent urinary tract infection. It is important to remember that crystal arthritis and infection may coexist and a thorough assessment of the synovial fluid is required to distinguish these conditions.

Acute benign joint effusions may occur in transplant recipients, in association with episodes of acute rejection. Chronic effusions are more difficult to treat and are often a consequence of mechanical factors. Intra-articular corticosteroid injection of the acute rather than chronic joint effusion is more likely to produce benefit.

Gout

Renal impairment, thiazides, ciclosporin, and tacrolimus are risk factors for gout. Two to 13% of post-transplant patients are affected (National Kidney Foundation, 2003; Stamp et al., 2005). Attacks may be acute or chronic, affecting peripheral joints, most commonly the first metatarsophalangeal joint. Serum urate is not always raised in an acute attack. Aspiration typically reveals negatively birefringent crystals under polarized light. Management includes intra-articular or oral steroids, with optional colchicine. NSAIDs should be avoided in those with renal impairment. Dietary measures to reduce purine consumption and altering antihypertensives can help prevent future attacks. Urosuric agents will be less effective in these patients. Low-dose colchicine and allopurinol are optional but caution should be taken with their use with ciclosporin (risk of myotoxicity) and azathioprine (risk of myelosuppression) respectively. Newer therapies are developing.

Calcium pyrophosphate deposition arthropathy/ pseudogout

Calcium pyrophosphate deposition (CPPD) can lead to acute synovitis. This is less common in patients with renal disease than urate or basic calcium pyrophosphate deposition disease, and is rare in dialysis patients. There is an association with Bartter syndrome. The disease can present as acute or monoarticular or pauci-articular arthritis (known as pseudogout), or as a chronic arthritis resembling degenerative joint disease (Ferrari, 1996).

The natural history of attacks is more variable than gout, and prolonged symptoms may occur partly due to the common association between CPPD disease and osteoarthritis. Predisposing factors/ associations include hyperparathyroidism, age, trauma, meniscectomy, hypomagnesaemia, hypothyroidism, hypophosphataemia, and haemochromatosis.

The treatment of coexisting conditions may have a variable impact on the arthritis. It is important to remember that infection also can coexist with the presence of CPPD in the synovial fluid. The therapy of this condition is limited; oral colchicine may be effective as a prophylaxis, but is not commonly used in patients with renal failure. Local intra-articular corticosteroids are the most effective and commonly used treatment, and have been shown to shorten acute attacks when compared to treatment with NSAIDs alone (O'Duffy, 1976).

Hydroxyapatite-related arthropathy

Basic calcium phosphate crystals can deposit in articular and peri-articular sites causing acute inflammatory episodes such as rotator cuff tendonitis, or a more chronic destructive arthropathy, such as Milwaukee shoulder. Corticosteroids and colchicine can be used in the acute setting, although chronic disease is difficult to treat. Physical therapy may be effective.

References

Adu, D., Berisa, F., Howie, A. J., *et al.* (1993). Glomerulonephritis in rheumatoid arthritis. *Br J Rheumatol*, 32, 1008–11.

Almirall, J., Alcorta, I., Botey, A., *et al.* (1993). Penicillamine-induced rapidly progressive glomerulonephritis in a patient with rheumatoid arthritis. *Am J Nephrol*, 13, 286–8.

Anders, H. J. and Vielhauer, V. (2011). Renal co-morbidity in patients with rheumatic diseases. *Arthritis Res Ther*, 13, 222.

Ansell, B. M., Eghtedari, A., and Bywaters, E. G. (1971). Chlorambucil in the management of juvenile chronic polyarthritis complicated by amyloidosis. *Ann Rheum Dis*, 30, 331.

Anttila, R. (1972). Renal involvement in juvenile rheumatoid arthritis. A clinical and histopathological study. *Acta Paediatr Scand Suppl*, 227, 3–73.

Anttila, R. and Laaksonen, A. L. (1969). Renal disease in juvenile rheumatoid arthritis. *Acta Rheumatol Scand*, 15, 99–111.

Arlen, D. J. and Adachi, J. D. (1999). Are bisphosphonates useful in the management of corticosteroid induced osteoporosis in transplant patients? *J Nephrol*, 12, 5–8.

Baggenstoss, A. H. and Rosenberg, E. F. (1943). Visceral lesions associated with chronic infectious (rheumatoid) arthritis. *Arch Pathol*, 35, 503–16.

Beaman, M., Adu, D., Howie, A. J., *et al.* (1987). Rheumatoid arthritis and IgA nephropathy. *Br J Rheumatol*, 26, 299–302.

Berg, K. J., Forre, O., Djoseland, O., *et al.* (1989). Renal side effects of high and low cyclosporin A doses in patients with rheumatoid arthritis. *Clin Nephrol*, 31, 232–8.

Boers, M., Croonen, A. M., Dijkmans, B. A., *et al.* (1987). Renal findings in rheumatoid arthritis: clinical aspects of 132 necropsies. *Ann Rheum Dis*, 46, 658–63.

Brun, C., Olsen, T. S., Raaschou, F., *et al.* (1965). Renal biopsy in rheumatoid arthritis. *Nephron*, 2, 65–81.

Buch, M. H., Smolen, J. S., Betteridge, N., *et al.* (2011). Updated consensus statement on the use of rituximab in patients with rheumatoid arthritis. *Ann Rheum Dis*, 70, 909–20.

Burdt, M. A., Hoffman, R. W., Deutscher, S. L., *et al.* (1999). Long-term outcome in mixed connective tissue disease: longitudinal clinical and serologic findings. *Arthritis Rheum*, 42, 899–909.

Chen, Y., Zhao, X., Tang, D., *et al.* (2010). IgA nephropathy in two patients with Sjogren's syndrome: one with concomitant autoimmune hepatitis. *Intern Med*, 49, 37–43.

Chiu, K. C., Tsai, T. C., Lin, W. T., *et al.* (2008). Paraneoplastic polymyositis associated with crescentic glomerulonephritis. *Ren Fail*, 30, 939–42.

Cobb, S., Anderson, F., and Bauer, W. (1953). Length of life and cause of death in rheumatoid arthritis. *N Engl J Med*, 249, 553–6.

Cohen, D. J. and Appel, G. B. (1992). Cyclosporine: nephrotoxic effects and guidelines for safe use in patients with rheumatoid arthritis. *Semin Arthritis Rheum*, 21, 43–8.

Cush, J. J., Tugwell, P., Weinblatt, M., *et al.* (1999). US consensus guidelines for the use of cyclosporin A in rheumatoid arthritis. *J Rheumatol*, 26, 1176–86.

Daoussis, D., Panoulas, V., Toms, T., *et al.* (2009). Uric acid is a strong independent predictor of renal dysfunction in patients with rheumatoid arthritis. *Arthritis Res Ther*, 11, R116.

Dass, S., Bowman, S. J., Vital, E. M., *et al.* (2008). Reduction of fatigue in Sjogren syndrome with rituximab: results of a randomised, double-blind, placebo-controlled pilot study. *Ann Rheum Dis*, 67, 1541–4.

David, J., Vouyiouka, O., Ansell, B. M., *et al.* (1993). Amyloidosis in juvenile chronic arthritis: a morbidity and mortality study. *Clin Exp Rheumatol*, 11, 85–90.

Dussol, B., Tsimaratos, M., Bolla, G., *et al.* (1994). [Crescentic glomerulonephritis and primary Gougerot-Sjogren syndrome]. *Nephrologie*, 15, 295–8.

Dwarakanath, A. D., Michael, J., and Allan, R. N. (1992). Sulphasalazine induced renal failure. *Gut*, 33, 1006–7.

Emery, P. and Panayi, G. S. (1989). Autoimmune reactions to D-penicillamine In M. E. Kanmuller, N. Bloksma, and W. Seinan (eds.) *Autoimmunity and Toxicology*, pp. 167–82. Amsterdam: Elsevier.

Emmungil, H., Kalfa, M., Zihni, F. Y., *et al.* (2012). Interstitial cystitis: a rare manifestation of primary Sjogren's syndrome, successfully treated with low dose cyclosporine. *Rheumatol Int*, 32, 1215–8.

Epstein, S., Shane, E., and Bilezikian, J. P. (1995). Organ transplantation and osteoporosis. *Curr Opin Rheumatol*, 7, 255–61.

Falck, H. M., Tornroth, T., Kock, B., *et al.* (1979). Fatal renal vasculitis and minimal change glomerulonephritis complicating treatment with penicillamine. Report on two cases. *Acta Med Scand*, 205, 133–8.

Ferrari, R. (1996). Rheumatologic manifestations of renal disease. *Curr Opin Rheumatol*, 8, 71–6.

Filipowicz-Sosnowska, A. M., Roztropowicz-Denisiewicz, K., Rosenthal, C. J., *et al.* (1978). The amyloidosis of juvenile rheumatoid arthritis—comparative studies in Polish and American children. I. Levels of serum SAA protein. *Arthritis Rheum*, 21, 699–703.

Fingerman, D. L. and Andrus, F. C. (1943). Visceral lesions associated with rheumatoid arthritis. *Ann Rheum Dis*, 3, 168–81.

Fournier, A., Nony, A., and Rifard, K. (2009). [Antineutrophil cytoplasmic antibody associated vasculitis in a patient treated with adalimumab for a rheumatoid arthritis]. *Nephrol Ther*, 5, 652–7.

Frost, N. A., Morand, E. F., Hall, C. L., *et al.* (1993). Idiopathic polymyositis complicated by arthritis and mesangial proliferative glomerulonephritis: case report and review of the literature. *Br J Rheumatol*, 32, 929–31.

Galesic, K., Prkacin, I., Tisljar, M., *et al.* (2009). [Renal involvement in patients with rheumatoid arthritis]. *Reumatizam*, 56, 30–5.

Gibson, T., Burry, H. C., and Ogg, C. (1976). Letter: Goodpasture syndrome and D-penicillamine. *Ann Intern Med*, 84, 100.

Goules, A., Masouridi, S., Tzioufas, A. G., *et al.* (2000). Clinically significant and biopsy-documented renal involvement in primary Sjogren syndrome. *Medicine*, 79, 241–9.

Grotz, W. H., Breitenfeldt, M. K., Braune, S. W., *et al.* (2001). Calcineurin-inhibitor induced pain syndrome (CIPS): a severe disabling complication after organ transplantation. *Transplant Int*, 14, 16–23.

Grotz, W. H., Mundinger, F. A., Rasenack, J., *et al.* (1995). Bone loss after kidney-transplantation—a longitudinal-study in 115 graft recipients. *Nephrol Dial Transplant*, 10, 2096–100.

Hall, C. L., Fothergill, N. J., Blackwell, M. M., *et al.* (1987). The natural course of gold nephropathy: long term study of 21 patients. *Br Med J*, 295, 745–8.

Hall, C. L., Jawad, S., Harrison, P. R., *et al.* (1988). Natural course of penicillamine nephropathy: a long term study of 33 patients. *Br Med J*, 296, 1083–6.

Helin, H., Korpela, M., Mustonen, J., *et al.* (1986). Mild mesangial glomerulopathy—a frequent finding in rheumatoid arthritis patients with hematuria or proteinuria. *Nephron*, 42, 224–30.

Helliwell, P. S. (1995). Should tests for proteinuria be included in the monitoring schedule of sulphasalazine? *Br J Rheumatol*, 34, 790–1.

Hengstman, G. J., Van Engelen, B. G., and Van Venrooij, W. J. (2004). Myositis specific autoantibodies: changing insights in pathophysiology and clinical associations. *Curr Opin Rheumatol*, 16, 692–9.

Honkanen, E., Tornroth, T., Pettersson, E., *et al.* (1987). Membranous glomerulonephritis in rheumatoid arthritis not related to gold or D-penicillamine therapy: a report of four cases and review of the literature. *Clin Nephrol*, 27, 87–93.

Hordon, L. D., Sellars, L., Morley, A. R., *et al.* (1984). Haematuria in rheumatoid arthritis: an association with mesangial glomerulonephritis. *Ann Rheum Dis*, 43, 440–3.

Immonen, K., Finne, P., Gronhagen-Riska, C., *et al.* (2011). A marked decline in the incidence of renal replacement therapy for amyloidosis associated with inflammatory rheumatic diseases—data from nationwide registries in Finland. *Amyloid*, 18, 25–8.

Immonen, K., Savolainen, A., Kautiainen, H., *et al.* (2008). Longterm outcome of amyloidosis associated with juvenile idiopathic arthritis. *J Rheumatol*, 35, 907–12.

Ito, S., Nakamura, T., Kurosawa, R., *et al.* (2006). Glomerulonephritis in children with mixed connective tissue disease. *Clin Nephrol*, 66, 160–5.

Kaneko, K., Nanki, T., Hosoya, T., *et al.* (2010). Etanercept-induced necrotizing crescentic glomerulonephritis in two patients with rheumatoid arthritis. *Mod Rheumatol*, 20, 632–6.

Karstila, K., Korpela, M., Sihvonen, S., *et al.* (2007). Prognosis of clinical renal disease and incidence of new renal findings in patients with rheumatoid arthritis: follow-up of a population-based study. *Clin Rheumatol*, 26, 2089–95.

Kassan, S. S., Thomas, T. L., Moutsopoulos, H. M., *et al.* (1978). Increased risk of lymphoma in sicca syndrome. *Ann Intern Med*, 89, 888–92.

Kaufman, I., Schwartz, D., Caspi, D., *et al.* (2008). Sjogren's syndrome—not just Sicca: renal involvement in Sjogren's syndrome. *Scand J Rheumatol*, 37, 213–18.

Kim, H. W., Choi, J. R., Jang, S. J., *et al.* (2005). Recurrent rhabdomyolysis and myoglobinuric acute renal failure in a patient with polymyositis. *Nephrol Dial Transplant*, 20, 2255–8.

Kitaura, K., Miyagawa, T., Asano, K., *et al.* (2006). Mixed connective tissue disease associated with MPO-ANCA-positive polyangiitis. *Intern Med*, 45, 1177–82.

Kitridou, R. C., Akmal, M., Turkel, S. B., *et al.* (1986). Renal involvement in mixed connective tissue disease: a longitudinal clinicopathologic study. *Semin Arthritis Rheum*, 16, 135–45.

Klouda, P. T., Manos, J., Acheson, E. J., *et al.* (1979). Strong association between idiopathic membranous nephropathy and HLA-DRW3. *Lancet*, 2, 770–1.

Koivuniemi, R., Paimela, L., and Leirisalo-Repo, M. (2009). Causes of death in patients with rheumatoid arthritis from 1971 to 1991 with special reference to autopsy. *Clin Rheumatol*, 28, 1443–7.

Korpela, M., Mustonen, J., Helin, H., *et al.* (1990). Immunological comparison of patients with rheumatoid arthritis with and without nephropathy. *Ann Rheum Dis*, 49, 214–8.

Kroot, E. J., Van Leeuwen, M. A., Van Rijswijk, M. H., *et al.* (2000). No increased mortality in patients with rheumatoid arthritis: up to 10 years of follow up from disease onset. *Ann Rheum Dis*, 59, 954–8.

Laakso, M., Mutru, O., Isomaki, H., *et al.* (1986). Mortality from amyloidosis and renal diseases in patients with rheumatoid arthritis. *Ann Rheum Dis*, 45, 663–7.

Lee, J. C., Dushkin, M., Eyring, E. J., *et al.* (1965). Renal lesions associated with gold therapy; light and electron microscopic studies. *Arthritis Rheum*, 8, 1–13.

Lin, D. F., Yan, S. M., Zhao, Y., *et al.* (2010). Clinical and prognostic characteristics of 573 cases of primary Sjogren's syndrome. *Chin Med J*, 123, 3252–7.

Maetzel, A., Wong, A., Strand, V., *et al.* (2000). Meta-analysis of treatment termination rates among rheumatoid arthritis patients receiving disease-modifying anti-rheumatic drugs. *Rheumatology*, 39, 975–81.

Mann, H. F., Vencovsky, J., and Lundberg, I. E. (2010). Treatment-resistant inflammatory myopathy. *Best Pract Res Clin Rheumatol*, 24, 427–40.

Maripuri, S., Grande, J. P., Osborn, T. G., *et al.* (2009). Renal involvement in primary Sjogren's syndrome: a clinicopathologic study. *Clin J Am Soc Nephrol*, 4, 1423–31.

Matsumura, R., Umemiya, K., Kagami, M., *et al.* (1995). [Immunohistochemical identification of infiltrating mononuclear cells in tubulointerstitial nephritis associated with Sjogren's syndrome]. *Nihon Rinsho*, 53, 2503–9.

Meijer, J. M., Meiners, P. M., Vissink, A., *et al.* (2010). Effectiveness of rituximab treatment in primary Sjogren's syndrome: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum*, 62, 960–8.

Meys, E., Devogelaer, J. P., Geubel, A., *et al.* (1992). Fever, hepatitis and acute interstitial nephritis in a patient with rheumatoid arthritis. Concurrent manifestations of azathioprine hypersensitivity. *J Rheumatol*, 19, 807–9.

Missen, G. A. and Taylor, J. D. (1956). Amyloidosis in rheumatoid arthritis. *J Pathol Bacteriol*, 71, 179–92.

Mutru, O., Koota, K., and Isomaki, H. (1976). Causes of death in autopsied RA patients. *Scand J Rheumatol*, 5, 239–40.

Nakano, M., Ueno, M., Nishi, S., *et al.* (1998). Analysis of renal pathology and drug history in 158 Japanese patients with rheumatoid arthritis. *Clin Nephrol*, 50, 154–60.

Nanra, R. S. (1975). Renal papillary necrosis in rheumatoid arthritis. *Med J Aust*, 1, 194–7.

National Kidney Foundation (2003). K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis*, 42, S1–201.

Nimelstein, S. H., Brody, S., McShane, D., *et al.* (1980). Mixed connective tissue disease: a subsequent evaluation of the original 25 patients. *Medicine*, 59, 239–48.

O'DuffY, J. D. (1976). Clinical studies of acute pseudogout attacks: comments on prevalence, predispositions, and treatment. *Arthritis Rheum*, 19 Suppl 3, 349–52.

Orjavik, O., Brodwall, E. K., Oystese, B., *et al.* (1981). A renal biopsy study with light and immunofluorescent microscopy in rheumatoid arthritis. *Acta Med Scand Suppl*, 645, 9–14.

Palmer, S. C., McGregor, D. O., and Strippoli, G. F. (2007). Interventions for preventing bone disease in kidney transplant recipients. *Cochrane Database Syst Rev*, CD005015.

Perez-Ruiz, F. and Nolla, J. M. (2003). Influence of leflunomide on renal handling of urate and phosphate in patients with rheumatoid arthritis. *J Clin Rheumatol*, 9, 215–18.

Pertovaara, M., Korpela, M., Kouri, T., *et al.* (1999). The occurrence of renal involvement in primary Sjogren's syndrome: a study of 78 patients. *Rheumatology*, 38, 1113–20.

Pollak, V. E., Pirani, C. L., Steck, I. E., *et al.* (1962). The kidney in rheumatoid arthritis: studies by renal biopsy. *Arthritis Rheum*, 5, 1–9.

Prior, P., Symmons, D. P., Scott, D. L., *et al.* (1984). Cause of death in rheumatoid arthritis. *Br J Rheumatol*, 23, 92–9.

Ramirez, G., Lambert, R., and Bloomer, H. A. (1981). Renal pathology in patients with rheumatoid arthritis. *Nephron*, 28, 124–6.

Rasker, J. J. and Cosh, J. A. (1981). Cause and age at death in a prospective-study of 100 patients with rheumatoid-arthritis. *Ann Rheum Dis*, 40, 115–20.

Ren, H., Wang, W. M., Chen, X. N., *et al.* (2008). Renal involvement and followup of 130 patients with primary Sjogren's syndrome. *J Rheumatol*, 35, 278–84.

Rifkin, S. I., Gutta, H., Nair, R., *et al.* (2011). Collapsing glomerulopathy in a patient with mixed connective tissue disease. *Clin Nephrol*, 75 Suppl 1, 32–6.

Salomon, M. I., Gallo, G., Poon, T. P., *et al.* (1974). The kidney in rheumatoid arthritis. A study based on renal biopsies. *Nephron*, 12, 297–310.

Sawai, T., Murakami, K., and Kurasono, Y. (1994). Morphometric analysis of the kidney lesions in mixed connective tissue disease (MCTD). *Tohoku J Exp Med*, 174, 141–54.

Schnitzer, T. J. and Ansell, B. M. (1977). Amyloidosis in juvenile chronic polyarthritis. *Arthritis Rheum*, 20, 245–52.

Sellars, L., Siamopoulos, K., Wilkinson, R., *et al.* (1983). Renal biopsy appearances in rheumatoid disease. *Clin Nephrol*, 20, 114–20.

Sharp, G. C., Irvin, W. S., Tan, E. M., *et al.* (1972). Mixed connective tissue disease—an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am J Med*, 52, 148–59.

Shibata, S., Ubara, Y., Sawa, N., *et al.* (2004). Severe interstitial cystitis associated with Sjogren's syndrome. *Intern Med*, 43, 248–52.

Singsen, B. H., Swanson, V. L., Bernstein, B. H., *et al.* (1980). A histologic evaluation of mixed connective tissue disease in childhood. *Am J Med*, 68, 710–7.

Skopouli, F. N. (2001). Kidney injury in Sjogren's syndrome. *Nephrol Dial Transplant*, 16 Suppl 6, 63–4.

Smolen, J. S. and Emery, P. (2011). Infliximab: 12 years of experience. *Arthritis Res Ther*, 13 Suppl 1, S2.

Sokka, T., Abelson, B., and Pincus, T. (2008). Mortality in rheumatoid arthritis: 2008 update. *Clin Exp Rheumatol*, 26, S35–61.

Stamp, L., Searle, M., O'Donnell, J., *et al.* (2005). Gout in solid organ transplantation: a challenging clinical problem. *Drugs*, 65, 2593–611.

Stetter, M., Schmidl, M., and Krapf, R. (1994). Azathioprine hypersensitivity mimicking Goodpasture's syndrome. *Am J Kidney Dis*, 23, 874–7.

Suzuki, A., Ohosone, Y., Obana, M., *et al.* (1994). Cause of death in 81 autopsied patients with rheumatoid arthritis. *J Rheumatol*, 21, 33–6.

Takizawa, Y., Kanda, H., Sato, K., *et al.* (2007). Polymyositis associated with focal mesangial proliferative glomerulonephritis with depositions of immune complexes. *Clin Rheumatol*, 26, 792–6.

Talar-Williams, C., Hijazi, Y. M., Walther, M. M., *et al.* (1996). Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener granulomatosis. *Ann Intern Med*, 124, 477–84.

Van De Merwe, J. P. (2007). Interstitial cystitis and systemic autoimmune diseases. *Nat Clin Pract Urol*, 4, 484–91.

Vincenti, F., Amend, W. J., Jr., Feduska, N. J., *et al.* (1982). Septic arthritis following renal transplantation. *Nephron*, 30, 253–6.

Voulgarelis, M., Tzioufas, A. G., and Moutsopoulos, H. M. (2008). Mortality in Sjogren's syndrome. *Clin Exp Rheumatol*, 26, S66–71.

Williams, H. J., Ward, J. R., Reading, J. C., *et al.* (1983). Low-dose D-penicillamine therapy in rheumatoid arthritis. A controlled, double-blind clinical trial. *Arthritis Rheum*, 26, 581–92.

Wolfe, F., Mitchell, D. M., Sibley, J. T., *et al.* (1994). The mortality of rheumatoid arthritis. *Arthritis Rheum*, 37, 481–94.

Yang, M. L., Kuo, M. C., Ou, T. T., *et al.* (2011). Primary Sjogren's syndrome with minimal change disease—a case report. *Kaohsiung J Med Sci*, 27, 190–4.

CHAPTER 167

The patient with sickle cell anaemia

Jean-Philippe Haymann and Francois Lionnet

Introduction and epidemiology

James Herrick's first description of sickle cell disease in 1910 included renal involvement: 'The urine was amber in color, specific gravity 1.010 to 1.014, slightly increased in amount—2,000 c.c.—acid, contained a distinct trace of serum-albumin, a few granular and hyaline casts. This represents the average of several examinations.' Indeed, a renal concentrating defect has been historically a matter of interest, together with renal papillary necrosis and renal failure. Renal failure is a recognized factor of mortality as assessed both by an autopsy study (Manci et al., 2003) and epidemiological surveys (Powars et al., 1991; Platt et al., 1994). The improvement in life expectancy from 14.3 years of age in 1973 up to 50 years in the 1990s (Wierenga et al., 2001) has raised attention to renal failure in this population as end-stage renal disease (ESRD) develops in 4.2–11.6% of adults with a median age of 37 years (Powars et al., 1991, 1995).

Sickle cell anaemia (SCA) (also called homozygous sickle cell disease or HbSS disease) is one of the more frequent genetic diseases in the world, particularly in sub-Saharan African populations. The annual number of birth has been recently estimated to be 217,331 for SCA, 54,736 for SC, and 11,074 for S-beta (β) thalassaemia (Weatherall, 2010). It is an autosomal recessive disorder due to a point mutation in the β-globin gene of haemoglobin (short arm of chromosome 11). This mutation is responsible for the synthesis of an abnormal haemoglobin protein called haemoglobin S (HbS) (made from a combination of two mutated β-globin chains with two alpha (α)-globin chains and haem), which polymerize in hypoxic conditions (deoxyhaemoglobin), leading to a sickling shape of red cells, a loss of plasticity, and ultimately vaso-occlusion and haemolysis.

In sickle cell disease, of the two inherited haemoglobin β-globin gene alleles, at least one must be abnormal (HbS). Thus, sickle cell disease entails some genetic heterogeneity within haemoglobin genes: SCA, characterized by two mutated β-globin alleles (HbSS), is encountered with the highest prevalence; and HbSC disease and HbS/β-thalassemia with a quantitative mutation that results in the absence of the β-globin gene (and thus no detectable HbA proteins), called HbS/β^0-thalassemia) or in reduced level of β-globin (called HbS/β^+-thalassemia)

Moreover, the sickle gene is multicentric with five identified main haplotypes (Rees et al., 2010): Arab-India, Benin, Senegal, Cameroon, and Bantu, which account in part for the severity of the disease despite an identical point mutation in the β-globin gene of haemoglobin.

For a given individual, the severity of the disease may be different during their lifetime with known and unknown genetic and environmental factors which may have a significant influence (such as α-thalassemia trait, hereditary persistence of fetal haemoglobin, cold weather, etc.). Thus, the broad range of clinical expression of this multisystem disease appears to clinicians as a random association of different acute events (such as painful vaso-occlusive crisis, acute chest syndrome, anaemia, and susceptibility to infections) and chronic organ complications (such as cerebral vasculopathy, osteonecrosis, retinopathy, pulmonary arterial hypertension, leg ulcer, priapism, and nephropathy) (Rees et al., 2010).

Homozygous sickle cell anaemia and kidney

Sickle cell-associated nephropathy

Introduction

A significant improvement in life survival has shifted medical focus on long-term complications such as chronic kidney disease (CKD). Indeed, prevalence of renal failure was reported in up to 85% of Jamaican SCA patients aged > 60 years (Serjeant et al., 2007), accounting for 43% of all causes of death (Serjeant et al., 2009). In young adult SCA patients, CKD was recently reported in 26% of cases, mainly stages 1 and 2 (Guasch et al., 2009). Although many issues about sickle cell-associated nephropathy (SCAN) remain unsolved, in particular, SCAN natural history, the focus on microalbuminuria and hyperfiltration which are considered as an early stage of SCAN have brought to light some interesting pathophysiological processes that could also be potential relevant determinants for CKD progression in the general population.

Hyperfiltration

Prevalence

Glomerular hyperfiltration has been an early sign in SCA children and adults (Etteldorf et al., 1952; Etteldorf et al., 1955). However, these data were not a main clinical issue in the 1950s as SCA child mortality was overwhelming. Recently, the very low plasma creatinine values detected by enzymatic in place of Jaffe methods brought some confusion, as an increased tubular creatinine secretion was previously reported in SCA (Allon, 1990; Herrera et al., 2002; Thompson et al., 2007). Nevertheless, an increased glomerular filtration rate (GFR) was assessed in several studies using GFR measurement techniques such as creatinine clearance (Alvarez et al., 2006), inulin clearance (Hatch et al., 1970; Allon 1990; Guasch et al., 1997; Hererra et al., 2002), plasma ^{51}Cr-ethylenediaminetetraacetic acid

Table 167.1 Comparison between different eGFR and mGFR methods relative to urinary ^{51}Cr EDTA mGFR to assess the prevalence of hyperfiltration status among 48 SCA patients with proteinuria

	Hyperfiltration, %	GFR, mL/min per 1.73 m^2	Se, %	Sp, %	PP, %
MDRD	72	161 (44–300)	94	63	82
Cockcroft and Gault	45	135 (65–320)	58	69	79
Creatinine clearance	62	144 (64–285)	87	88	90
Plasmatic mGFR	74	121 (62–171)	97	69	89
Urinary mGFR	66	123 (54–188)	100	100	100

Se: sensitivity; Sp: specificity; PP: predictive power.

(EDTA) clearance (Thompson et al., 2007), renal ^{51}Cr-EDTA clearance (Haymann et al., 2010), or plasma ^{99m}Tc-diethylenetriamine pentaacetate (DTPA) clearance (Ware et al., 2010), though in most cases hyperfiltration prevalence was not addressed precisely in a non-selected SCA population. Of note, the magnitude of hyperfiltration was very high for some individuals with measured GFR (mGFR) values > 200 mL/min/1.73 m^2 (Schmitt et al., 1998; Alvarez et al., 2006; Thompson et al., 2007; Ware et al., 2010). The high incidence of hyperfiltration in young adults was assessed recently using a 140 mL/min/1.73 m^2 cut off value (Modification of Diet in Renal Disease (MDRD) Study estimated GFR (eGFR)) after validation on a subgroup of SCA patients with renal ^{51}Cr EDTA clearance (measured GFR > 110 mL/min/1.73 m^2) (Table 167.1). Using this threshold, prevalence of hyperfiltration was between 50% and 60% in SCA patients (Haymann et al., 2010; Day et al., 2012).

In child SCA populations, a reported high eGFR (> 150 mL/min/1.73 m^2) using the Schwartz formula (Schwartz, 1987) was described in several reports with an age-related increase in the first decade of life (Dharnidharka et al., 1998; Wigfall et al., 2000). Hyperfiltration prevalence increases with age (assessed by plasma ^{99m}Tc-DTPA clearance), ranging from 58% (9–12 months of age) to 78% (after 15 months) (Ware et al., 2010); GFR decreases occur after the age of 16 years (Aygun et al., 2011).

Determinants of hyperfiltration

In our young adult SCA population (mean age 25.8 years old), only 20% of the patients were free of renal disease, hyperfiltration being considered as a renal involvement (Etteldorf et al., 1955; Schmitt et al., 1998), thus raising the issue of the determinants accounting for this pathological state. Glomerular hyperfiltration pathophysiology is a matter of great interest in type 1 diabetic patients and is currently viewed as an increased renal plasma flow, and glomerular hydraulic pressure leading to increased filtration fraction (FF) (Anderson and Vora, 1995), altogether with increased glomerular permeability and Kf values. SCAN pathological processes seem different as the increased values of effective renal plasma flow (ERPF) in children and young adults is associated with a low FF (12–16%) (Allon, 1990; Guasch et al., 1997; Schmitt et al., 1998; Thompson et al., 2007). Low FF, explained by a proportionally greater ERPF rise compared to GFR, suggests a dilation of both afferent and efferent arterioles, with a predominant effect on the latter. These findings are in agreement with data reporting low intrarenal resistance (Guasch et al., 1997) and a lower mean systemic arterial pressure than a control population matched for ethnicity and age (Thompson et al., 2007).

Anaemia was suggested to explain increased cardiac output (Leight et al., 1954; Roy et al., 1963), and thus ERPF increase. However, a renal microvascular dyregulation specific to SCA was suspected as early as the 1970s since multiple transfusions with normal red blood cells had no effect on renal haemodynamic (Statius van Eps et al., 1967). This assessment was strengthened by the absence of significant renal haemodynamic effect reported in other types of anaemia, such as iron deficiency anaemia (Bradley et al., 1947).

Medullar vasa recta rarefaction was highlighted as a major factor accounting for renal haemodynamic dysregulation, and supported by convincing microradioangiographic studies (Fig. 167.1) (Statius van Eps et al., 1970). Indeed, the view that medullary abnormalities could be responsible for the increased cortical renal plasma flow and in some cases also for papillary necrosis was in agreement with the renal clinical phenotypes of SCA patients. This current view is challenged by the finding that prevalence of hyperfiltration is very low in patients with HbSC disease (< 6%) (Day et al., 2012; Lionnet et al., 2012) whereas vasa recta rarefaction is also present and generally considered to be related to vaso-occlusive processes (Statius van Eps et al., 1970). Though the magnitude of medullar vasa recta rarefaction by itself could explain various degree of tubuloglomerular feedback (TGF) dysregulation as demonstrated in experimental renal papillectomy (Takenaka et al., 1994; Ichihara et al., 1998), such renal haemodynamic features are probably the results of the interplay between several mediators including local prostaglandin, nitric oxide (NO), renin angiotensin II, endothelin, and/or adenosine release. The role of these mediators has remained under investigation for more than a decade with no comprehensive view to date. Our recent findings in young adult SCA patients suggest that hyperfiltration would be associated with a haemolysis rather than a vaso-occlusive phenotype, thus stressing the roles of two potential candidates: NO and carbon monoxide (CO) (Haymann et al., 2010) (see 'Sickle cell anaemia in mice: pathophysiological insights').

Micro- and macroalbuminuria

Prevalence

In paediatric SCA patients, microalbuminuria occurs as early as 7 years old (Dharnidharka et al., 1998) with a prevalence ranging between 12% and 28% (Aoki et al., 1990; Dharnidharka et al., 1998; Wigfall et al., 2000; McBurney et al., 2002; McKie et al., 2007; Marsenic et al., 2008; Alvarez et al., 2008; Becton et al., 2010; Gurkan et al., 2010; Aygun et al., 2011; King et al., 2011; McPherson Yee et al., 2011). In adult SCA patients, prevalence of albuminuria increases with age, ranging from 26% to 68% (depending also on the criteria used to assess albuminuria) (Guasch et al., 2006; Thompson et al., 2007; Haymann et al., 2010; Nebor et al., 2010;

Fig. 167.1 Microangiograph of a pyramid from (A) a normal kidney, (B) a HbSC disease kidney (5 years old), and (C) a HbSS disease kidney (3 years old).
From Statius van Eps et al. (1970).

Asnani et al., 2011; Day et al., 2011; Sharpe and Thein, 2011). The prevalence of proteinuria within the nephrotic range is low (< 4%) (Bakir et al., 1987; McPherson Yee et al., 2011; Sharpe and Thein 2011; and authors' unpublished data).

Data about the natural history of SCAN are lacking; however, the general view is the occurrence of micro- before macroalbuminuria as the prevalence of non-proteinuric patients decreases with ageing (40% in SCA between 18 and 30 years old, and 20% in SCA after 40 years old). Conversely, the prevalence of macroalbuminuria increases with ageing (20% in SCA between 18 and 30 years old, and 40% in SCA after 40 years old) (Guasch et al., 2006). The prevalence of albuminuria after the age of 40 is high, up to 80%. Of note, the prevalence of hyperfiltration alone or associated with microalbuminuria is about the same range whereas the prevalence of patients with hyperfiltration and macroalbuminuria is much lower (Fig. 167.2), favouring the view that hyperfiltration would be the first step of SCAN with no or minor ultrastructural damages, that could ultimately worsen and lead to a decreased GFR and the onset of macroalbuminuria at a later stage.

Determinants of albuminuria

Despite the fact that hyperfiltration is frequent and occurs very early in childhood in SCA patients and thus appears as a potential necessary condition for the onset of albuminuria, a direct link has not been fully demonstrated in SCA patients to date. The identification of relevant determinants responsible for albuminuria is still under debate.

Micro- and macroalbuminuria may be mediated by different factors corresponding to selective and non-selective proteinuria. Microalbuminuria in patients at the time of hyperfiltration have selective proteinuria, and an increased filtration coefficient (Kf) related to glomerular hypertrophy with a low filtration fraction (FF) (Schmitt et al., 1998). In agreement with this hypothesis, intermittent microalbuminuria has been described in up to 34% of SCA children (selected criteria: albumin:creatinine ratio > 30 mg/g) (Alvarez et al., 2008) in favour to a reversible state, that is, haemodynamic rather than histological lesions. Conversely, macroalbuminuria corresponds to the presence of non-selective proteinuria assessed by an increased dextran permeability clearance with an incremental increase in the pore radius (Guasch et al., 1997) and would reflect glomerular structural damages with a normal or increased FF suggesting glomerular capillary hypertension. This view is supported by the decreased albumin excretion rate observed after angiotensin-converting enzyme inhibitor (ACEI) administration (Falk et al., 1992) (see 'Therapeutic management').

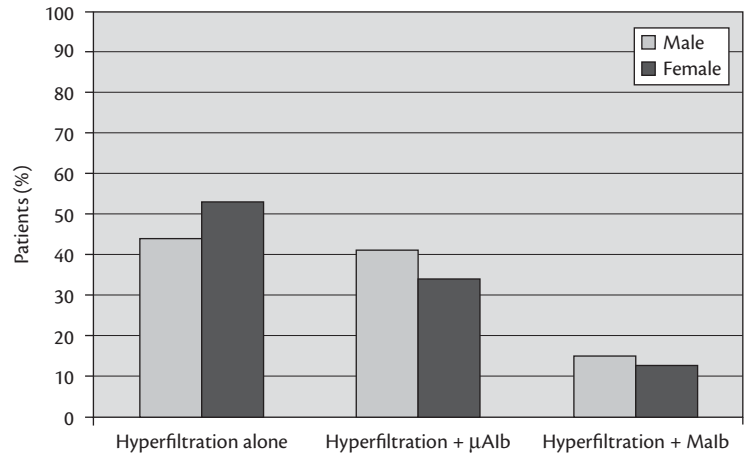

Fig. 167.2 Distribution of patients with SS disease with hyperfiltration according to albuminuria. µAlb = microalbuminuria; Malb =macroalbuminuria. N = 280.
From Haymann et al. (2010).

The stimulation of the local renin–angiotensin system is probably at play with little impact on systemic blood pressure, conversely to other nephropathies (including diabetes at the time of hyperfiltration). Indeed, despite a higher diastolic blood pressure in albuminuric compared to non-albuminuric patients (Bolarinwa et al., 2012), blood hypertension incidence is very low (2–6%) in the SCA population compared to the US black population where the incidence is 28% (Hatch et al., 1989; Saborio and Scheinman, 1999).

In the literature, no distinction is made to study the determinants of micro- and macroalbuminuria separately, and most of the time CKD patients are not excluded from the analysis, thus providing potential additional bias. A growing literature supports the view that haemolysis is associated with albuminuria based upon the demonstrated protective effect of α-thalassemia against SCAN (Nebor et al., 2010): coinheritance of α-thalassemia in SCA patients is known to be associated with a lower rate of haemolysis (Embury et al., 1982; Higgs et al., 1982). Indeed, in albuminuric SCA patients, prevalence of α-thalassemia is lower compared to normoalbuminuric (Guasch et al., 1999; Nebor et al., 2010), and in the adult SCA population, the age at onset of albuminuria is delayed compared to patients without α-thalassemia. Whereas intravascular haemolysis biomarkers such as lactate dehydrogenase (LDH) seem not to be strong predictors for albuminuria (Guasch et al., 2006), log(RBC-Hb/Ret-Hb), a marker of red cell lifespan, was reported to be lower in albuminuric SCA patients (Maier Redelsberger et al., 2010) with an even better link when associated into a composite variable including LDH and reticulocytes. This study has been confirmed recently by another team (Day et al., 2012). Nevertheless, the true determinants of albuminuria remain an open question.

Chronic renal failure

Prevalence

In a SCA paediatric cross-sectional study including 189 patients, CKD was present in 27% of patients including CKD stage 1 (15%) and stage 2 (12%) with no reported stage 3, 4, or 5 CKD (McPhersonYee et al., 2011). In our series of young adult SCA patients (N = 442), 8% had CKD stages 3–5, 68% were CKD stages 1 and 2, and 24% free of any renal pathology (authors' unpublished data).

However, if SCAN alone may progress to ESRD only in a few cases, the reported 4–12% SCA patients with ESRD (Powars et al., 1991, 2005) are related in most cases to nephrotoxic drugs administration, critical conditions in intensive care, and/or acute renal injury such as renal infarctions, papillary necrosis, pyelonephritis, or renal vein thrombosis. In SCA patients older than 60 years, prevalence of renal impairment rises up to 85% (Serjeant et al., 2007).

Determinants of CKD

Hypertension is present in 28% of SCA patients undergoing a renal biopsy (Maigne et al., 2010). Of note, several reports highlight unusual normal/high blood pressure values (systolic blood pressure 120–139 mmHg, diastolic 70–89 mmHg) compared to non-CKD SCA patients (Thompson et al., 2007; Gordeuk et al., 2008). Thus, values that would be considered normal in healthy individuals should be considered at risk for cardiovascular complications in patients with SCA with a positive association between blood pressure, stroke, pulmonary hypertension, and increased mortality (Pegelow et al., 1997; Gordeuk et al., 2008).

Other risk factors associated with CKD include severe anaemia, proteinuria, nephrotic syndrome, and microscopic haematuria (Bakir et al., 1987; Powars et al., 1991; Maigne et al., 2010).

Pathological features

Renal manifestation of SCA cover a wide array of lesions including focal areas of haemorrhage or necrosis, oedema, interstitial inflammation, fibrosis, tubular atrophy, and haemosiderin deposits. Medulla vasa recta rarefaction and maximally hypertrophied glomeruli with or without focal sclerosis are two striking features detected in most cases.

Vascular lesions

Haemoglobin polymerization is responsible for sickled red blood cell occlusion of blood flow in vasa recta resulting to typical medullary and papillary necrosis in 15–36% of cases (McCall et al., 1978).

Such vascular lesions within medulla and papilla are probably related to a low oxygen pressure, a hypertonic gradient, and an acidic environment which favour polymerization of SS haemoglobin (Mozzarelli et al., 1987). Glomerular capillary obliteration is also described at a later stage (Bhathena and Sondheimer, 1991) (see below).

Glomerular lesions

Glomerular hyperfiltration is characterized by a distinctive morphological pattern with glomerular enlargement, compared to control subjects (Bernstein and Whitten, 1960; Pitcock et al., 1970; Buckalew and Someren, 1974). In adult patients, glomerular size is increased by > 50% compared to controls (Elfenbein et al., 1974; Falk et al., 1992). In older patients with glomerular involvement, progressive ischaemia and fibrosis lead to glomerulosclerosis. However, whatever the magnitude of albuminuria, the size of the glomeruli is larger in SCA patients compared to other glomerulopathies such as idiopathic focal glomerulosclerosis or diabetes (Bhathena and Sondheimer, 1991; Osterby et al., 1992).

As kidney biopsies are not routinely performed in SCA patients with glomerular involvement, the prevalence of histological lesions is lacking and scarce reports may over-represent some findings and conversely under-represent other features. To our knowledge, three short series were reported before 2000 (Tejani et al., 1985; Bakir et al., 1987; Falk et al., 1992) and one larger series recently (Maigne et al., 2010), identifying noteworthy focal segmental glomerulosclerosis (FSGS) in 30–50% of cases (Mccoy et al., 1969; Buckalew and Someren, 1974), membranoproliferative glomerulonephritis (MPGN) in < 30%, and various lesions including thrombotic microangiopathy glomerulopathy in 17% but also glomerular hypertrophy alone, with no other glomerular lesions and immunoglobulin or complement deposits (Maigne et al., 2010). Of note, MPGN lesions were not related to hepatitis C or chronic bacterial infection, favouring the view that MPGN lesions could be related to SCA status. It is thus tempting to speculate that glomerular enlargement with capillaries distended by sickled erythrocytes could both explain hyperfiltration at early stage of the disease and the increased occurrence of a variety of immune and non-immune mediated glomerular lesions.

Tubulointerstitial lesions

Abundant haemosiderin granules in proximal tubular epithelial cells are reported in most biopsies (Bhathena and Sondheimer, 1991; Maigne et al., 2010) with various degrees of tubular atrophy and focal interstitial fibrosis in one series (Falk et al., 1992) and no inflammatory cells in another (Maigne et al., 2010). Focal necrosis, oedema, and extensive areas of fibrosis are reported in the papilla (Saborio and Scheinman, 1999). Renal dysfunction is reported to

be linked to tubular damage. In this regard, haemosiderin deposits should draw our attention as they may indicate an imbalance skewing to a pro-oxidant state within tubules (and possibly endothelial cells) which may explain tubular lesions.

Renal biopsy indication

Percutaneous renal biopsy can be performed when indicated after a complete coagulation profile and thus sometimes requires a red blood cell transfusion or exchange as a preconditioning. No available data report a higher prevalence of post-biopsy bleeding. Nevertheless, a renal biopsy can be avoided most of the time if SCAN is suspected, but remains mandatory in cases of acute kidney injury of unknown origin especially in the setting of high blood pressure. In our view, acute onset of nephrosis rules out a SCAN and would thus suggest several pathologies such as renal vein thrombosis, lupus, MPGN (related or not to hepatitis C or human immunodeficiency), infection, drug nephrotoxicity, thrombotic microangiopathy, and so on. Of note, a few acute nephrotic syndromes worsening a pre-existing albuminuria were reported to be linked to parvovirus B19 infections (Wierenga et al., 1995; Quek et al., 2010).

Other renal injuries

Papillary necrosis and medullary injuries

As previously mentioned, the prevalence of renal infarcts and papillary necrosis was estimated to be around 30–40% in the 1980s in populations where it was systematically searched by urography (McFall et al., 1978; Odita et al., 1983; Vaamonde, 1984). In most patients with SCA, the infarcted areas are small, and most of the time asymptomatic. However, papillary necrosis can present with renal colic, gross haematuria, urinary tract infection, or rarely acute kidney injury with urinary tract obstruction (Vaamonde, 1984). Degree of papillary necrosis would depend on several factors including intracellular haemoglobin concentration and higher levels of fetal haemoglobin or haemoglobin A_2.

Medullary microvascular impairment and papillary necrosis explain urinary concentrating ability impairment leading to hyposthenuria and ultimately polyuria which is < 5% in our cohort (unpublished data). An unravelled pathological link between SCA and papillary carcinoma is reported, especially with sickle cell trait (SCT) (see below) and should be distinguished from papillary necrosis (inactivation of tumour suppressor gene *SMARCB1* may be linked to renal medullary carcinoma) (Liu et al., 2013).

Acute kidney injury

Four to 10% of hospitalized SCA patients have acute kidney injury (Devereux and Knowles, 1985; Kelly and Singer, 1986; Skar et al., 1990; Hassell et al., 1994), often related to severe infections or rhabdomyolysis with occasional renal vein thrombosis. A statistical link to acute chest syndrome and pulmonary hypertension has been recently described (Audard et al., 2010). Few histological studies are available reporting ischaemic insults located in kidney cortex, including acute congestion of the glomerular microcirculation, cortical ischaemia, and cortical infarction (de Jong and Statius van Eps, 1985; Saborio and Scheinman,1999). In a paediatric population, acute kidney injury was associated with an older age, pre-existing hypertension, and haematuria (Stallworth et al., 2011). Among nephrotoxic drugs, non-steroidal anti-inflammatory agents and iron chelators may induce acute kidney injury (Schaller and Kaplan, 1998; Prasannan et al., 2003).

Tubulopathy

Tubular abnormalities affect mainly a reduced ability to concentrate urine. Maximum urine osmolality assessment is about 400–450 mOsm/kg of water (Levitt et al., 1960; Hatch, 1967; de Jong and Statius van Eps, 1985). An incomplete form of distal acidosis characterized by a normal or decreased ammonium excretion associated with an impaired titratable acid excretion is described (Saborio and Scheinman, 1999) altogether with potassium excretion impairment (DeFronzo et al., 1979; de Jong and Statius van Eps, 1985). No convincing explanations are provided to account for these electrolyte disorders, as plasma renin and aldosterone concentrations are generally normal. Conversely to other SCA populations (Saborio and Scheinman, 1999), in our series, the prevalence of mild metabolic acidosis (plasma bicarbonates between 17 and 23 mmol/l) is high (42% of cases) with no other current hypothesis than an impaired ammonium secretion. Hyperkalaemia encountered in < 2% of our population affects only CKD patients. Of note, an enhanced tubular reabsorption of phosphate leading in some cases to hyperphosphataemia or more often to upper limits is a common biological feature with no current clear explanation (De Jong, 1978). Tubular urate secretion is preserved (Morgan, 1984).

Pyelonephritis and urinary tract infection

SCA patients are predisposed to infection for several reasons, especially asplenia. The prevalence of urinary tract infections is high, probably due to local alterations such as papillary necrosis. Recurrent pyelonephritis is also frequently encountered in this population especially during pregnancy (Villers et al., 2008), triggering painful vaso-occlusive crises.

Therapeutic management

Hyperfiltration

One recent study suggests that hydroxyurea (hydroxycarbamide) treatment at maximum tolerated dose may normalize hyperfiltration. Twenty-three children (median age 7.5 years) receiving hydroxyurea for 3 years significantly decreased their GFR (measured by ^{99m}Tc-DTPA) from 167 ± 46 mL/min/1.73 m^2 to 145 ± 27 mL/min/1.73 m^2 strengthening the view that haemolysis decrease induced by hydroxyurea (assessed by fetal haemoglobin and LDH levels) could be efficient on hyperfiltration (Aygun et al., 2013). Conversely, in the BABY HUG trial, infants (average 13.6 months old) randomly allocated to receive either placebo or hydroxyurea therapy (at lower dose), no benefit in preventing hyperfiltration progression was observed after a 2-year follow-up (from 125 to 147 mL/min/1.73 m^2, using ^{99m}Tc-DTPA renal clearance) (Aygun et al., 2013).

Albuminuria

A few studies, including a limited number of patients, suggest that albuminuria could be either significantly reduced or even may disappear under ACEI therapy (Falk et al., 1992; Aoki et al., 1995; Foucan et al., 1998; Fitzhugh et al., 2005). Of note, short-term treatment for 2 weeks was reported to decrease proteinuria by 57% with no significant influence on GFR (Falk et al., 1992). ACEI treatment for 6 months was reported to normalize albuminuria in six out of eight patients (75%) in one non-controlled study (Aoki et al., 1995) and to decrease albuminuria significantly by 37% (compared to 17% in the placebo group) in a randomized study (Foucan et al., 1998). Of interest, in the latter study, no significant difference between the treated and placebo group were noticed after 1 month

of treatment. Recently, no beneficial effect of hydroxyurea was reported in a paediatric population (Alvarez et al., 2012).

Renal failure

SCAN may lead to a progressive GFR decrease associated with an increased albuminuria excretion rate revealing glomerulosclerosis (Powars et al., 1991; Bhathena and Sondheimer, 1991). This course is different from the acute onset of the nephrotic syndrome typically seen in idiopathic primary FSGS and minimal change disease (Nasr et al., 2006). No SCA-specific nephroprotection is proven though a general agreement seems to be to avoid nephrotoxic drugs and also to lower blood pressure which may be an important target in this setting where renal haemodynamic impairment is suspected.

Dialysis

Median life expectancy is 27 years for CKD patients as opposed to 51 years for those without renal failure. Although very few data are available, median survival after ESRD seems very poor (i.e. 4 years) (Powars et al., 1991) with 14% of patients alive at 10 years (Scheinman, 2009). Dialysis impacts significantly on survival with an increased mortality risk (hazard ratio 1.52). Of note, SCA patients are much less likely to be listed for or receive renal transplantation than other comparable patients with ESRD (US registry data; Abbott et al., 2002).

There is a general agreement that haemoglobin level > 10 g/dL could favour acute complications, thus suggesting a cautious use of erythropoietin therapy and red blood cell transfusions in this setting (Lionnet et al., 2009).

Transplantation

The survival rate for patients with SCA who have received a renal transplant is 56% at 10 years, with no significant difference between SCA and African American patients when age-matched controls were used (Scheinman, 2009). One-year graft survival is 78% and comparable with that in controls; however, graft survival at 3 years decreased significantly at 48% with an increased mortality rate (relative risk: 2.8) (Ojo et al., 1999). A recent study showed that patient survival has improved among contemporary sickle cell recipients (73.1%) compared with an earlier cohort and is now comparable to a matched cohort of diabetic kidney recipients (Huang et al., 2013). Graft loss-specific causes are related to acute vaso-occlusive crisis with graft thrombosis and early failure and recurrent sickle nephropathy (Donnely et al., 1988; Montgomery et al., 1994; Warady and Sullivan, 1998).

Sickle cell anaemia in mice: pathophysiological insights

Mice strains

Two main transgenic mice have been created expressing human haemoglobin with a *b56v* mutation (β^s-globins). The BERK (Berkeley) strain expresses exclusively human α- and β^s-globins with various levels of HbF (from 1% to 40%) allowing the influence of HbF on intravascular sickling and haemolysis (Dasgupta et al., 2010). The other is the SAD strain which harbours the mutated human β^s-globins with two additional mutations (Antilles b231 and D Punjab b121q) promoting an increase in polymer formation in the presence of mouse haemoglobin.

Renal phenotype

BERK strains exhibit features of human sickle cell nephropathy even at a relatively young age (Pászty et al., 1997; Ryan et al., 1997) whereas in SAD mice, glomerular hypertrophy and mesangial sclerosis develop in severity with age altogether with renal haemosiderosis, marked congestion in capillary loops, cortical peritubular and medulla capillaries with microvascular occlusions leading to cortical and papillary infarcts (De Paepe and Trudel, 1994; Nath et al., 2005). Glomerulomegaly is a striking feature in these animals compared to controls (Trudel et al., 1991; De Paepe and Trudel, 1994) raising the issue of a potential increase in plasma renal flow leading to a hyperfiltration state. Surprisingly, cardiac output and blood flow velocity in the renal arteries were similar in control and SAD mice (Sabaa et al., 2008). However, hyperfiltration was assessed in the first-generation transgenic animals (Fabry et al., 1995) by a measured GFR (25% increase using inulin clearance) and suggested by lower plasma creatinine levels using second-generation mice. Medullary hypoxia associated with vaso-occlusion was confirmed by renal magnetic resonance imaging, in accordance with a decreased concentrating ability reported in several strains (Fabry et al., 1995; Nath and Katusic, 2012).

Influence of hypoxia on renal haemodynamics

In a controlled, low-oxygen, partial pressure environment, mean renal blood flow and cardiac output of SAD mice are decreased with a marked renal vascular congestion (Bonnin et al., 2008; Sabaa et al., 2008). Similarly, in a renal ischaemia model, shortly after the release of the ischaemic clamp from the renal artery, renal plasma flow and GFR are markedly decreased in sickle mice compared to wild-type mice. Renal vascular resistance increases more than threefold (Juncos et al., 2010; de Franceschi et al., 1999), consistent with a vasoconstrictive myogenic reflex of kidney resistor vessels and/or erythrocyte congestion in small arteries, arterioles, glomerular capillaries, and medullary microcirculation. Of note, 24 hours following localized renal ischaemia, a prominent systemic inflammatory response in distant organs develops in sickle mice (Nath et al., 2005).

Several local mediators play a role in the protection of the medulla from ischaemic insults such as NO, and more recently the focus has been on CO. Contrary to endothelin, both molecules share the ability to enhance medullary blood flow and inhibit salt reabsorption along the nephron (Pallone, 2007). The regulation of these mediators in SCA mice kidneys will be briefly reviewed.

Evidence for a dysregulation of NO balance

An impaired NO bioavailability assessed in human SCA by a reduced flow-mediated vasodilation (Reiter et al., 2002; Eberhardt et al., 2003; Gladwin et al., 2003; Gladwin and Kato, 2005) has been also demonstrated in SCA models with an attenuated response to NO-mediated vasoactive stimuli (Kaul et al., 2000; Nath et al., 2000; Kaul and Fabry, 2004; Hsu et al., 2007). Under the conditions of chronic hypoxia and haemolysis, induction of non-NO vasodilators, such as prostaglandins (COX2, PGE_2) and haem oxygenase-1 (HO-1) may compensate reduced NO bioavailability (Graido-Gonzales et al., 1998; Gladwin et al., 2003; Kaul and Fabry, 2004; Nath et al., 2004) and help maintain optimal oxygen delivery in the face of chronic anaemia. However, an enhanced inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) expression is detected in SCA mice kidneys (Bank et al., 1996). These data are in accordance with the known vascular effect of medullary iNO production in maintaining perfusion and protecting the medulla from ischaemic injury altogether with its inhibition of salt reabsorption (in the thick ascending limb) and

water reabsorption (in the collecting duct) which both reduce local oxygen consumption. Intrarenal iNOS upregulation has thus been proposed to explain hyperfiltration and awaits further data in SCA patients.

Evidence for endothelin-1 involvement

In humans, endothelin-1 (ET-1) levels are abnormally high in plasma and urine from SCA patients in basal conditions (Rybicki and Benjamin, 1998) during acute complications such as chest syndromes (Hammerman et al., 1997) and chronic complications such as renal involvement (Tharault et al., 2005).

In SAD mice, high levels of ET-1 mRNA were detected in endothelium (Sabaa et al., 2008). SAD mice chronically treated with an ET inhibitor (bosentan) did not display significant haemodynamic changes at steady state whereas following a controlled hypoxia/reoxygenation the 50% decreased renal blood flow was reversed within minutes by acute ET receptor antagonists. These data favour the view that ET-1 within kidneys may sustained a triggered vasoconstrictive response and thus participate in organ injury and mortality in response to hypoxia reoxygenation (Sabaa et al., 2008), but does not provide a straightforward clue for hyperfiltration mechanism.

Evidence for haem/haem oxygenase involvement

In SCA patients, chronic haemolysis is responsible for haem and free haemoglobin release that upregulate several genes coding for haem metabolism including HO-1 both in kidney and circulating endothelial cells (Nath, 2001; Bains et al., 2010). HO-1 is an essential enzyme for haem degradation recruited by the vasculature for cytoprotection against high haem exposure (Balla et al., 2007) and catalyses the enzymatic degradation of free haem/haemoglobin to free iron, biliverdin (subsequently reduced to bilirubin), and CO (Kharitonov et al., 1995). Upregulation of HO-1 was also reported in SCA mice kidneys, especially in vessels (Nath et al., 2001; Juncos et al., 2010). HO-1 would be an interesting candidate to explain hyperfiltration as HO induction attenuates an afferent arteriolar autoregulatory myogenic reflex thus modulating renal blood flow (Botros et al., 2008; Wang et al., 2010). Of interest, endogenously produced CO would exert a renoprotective reserve mechanism to prevent excess afferent arteriolar constriction in conditions where NO production is inhibited or angiotensin II stimulated (Botros et al., 2006; Nath et al., 2010).

HO-1 in the setting of chronic haemolysis also emerges as a key player in the skewing of pro-oxidant haem and iron versus anti-oxidant CO, biliverdin, and bilirubin thus potentially preventing kidney inflammation and other SCA complications (Belcher et al., 2006; Nath and Katusic 2012).

Other major sickle cell diseases and kidney

Very few data related to renal disease are available in non-homozygous sickle cell disease patients. S/β^0-thalassemia patients share the same phenotype than SCA, whereas SC and S/β^+-thalassemia have a different expression. These haemoglobinopathies are characterized by higher haemoglobin concentrations suggesting that hyperviscosity may be a relevant factor. One striking difference between HbSC and SCA patients is the lower prevalence of renal involvement, with very few glomerular abnormalities (Table 167.2). SCAN, defined as the association of glomerular hyperfiltration and albuminuria, is exceptional, whereas comorbidities such as hypertension, diabetes, or HIV infection are more prevalent in this HbSC population with renal involvement (Lionnet et al., 2012).

Table 167.2 Prevalence of renal involvement in SC patients compared to SS patients (authors' unpublished data)

Condition	HbSC No. (%)	SCA %
No renal involvement	151 (87)	16
Glomerular hyperfiltration	9 (5)	51
Microalbuminuria	9 (5)	40
Macroalbuminuria	4 (2)	19
Renal insufficiency	4 (2)	7

Sickle cell trait and kidney

SCT prevalence is high (300 million worldwide), especially in West Africa. SCT is characterized by the inheritance of a normal haemoglobin gene (HbA) from one parent and an abnormal, mutated β-globin gene, the sickle haemoglobin gene (HbS), from the other parent. This status, described as a benign carrier state with a life survival similar to the general population (Ashcroft at al 1976), may nevertheless, during hypoxic circumstances or dehydration episodes, lead to acute kidney injury, spleen infarction, venous thrombosis, and sudden death (Tsaras et al., 2009). Genetic advice is currently given in Western countries when both parents are heterozygous for HbS. Among population with a high prevalence for SCT, a systematic genetic evaluation before renal transplantation both for recipients and living donors is an ongoing debate (see below).

Renal involvement

As previously mentioned, the low oxygen content of the renal medulla provides a propitious setting for intravascular sickling. In fact, HbAS has been associated with fewer and disrupted vessels of the vasa recta and renal microvascular obstruction (Statius van Eps et al., 1970), which may thus account for several complications reported in the literature with a low prevalence (Table 167.3). A potential link between SCT and chronic renal failure was raised in a retrospective study in ESRD patients showing that HbAS was twice as common among African Americans with ESRD compared with the general African American population (15% vs 7%) (Derebail et al., 2010). Such an association was not confirmed by another study (Hicks et al., 2011) and thus requires a prospective study.

Renal transplantation

The consequences for donor and receiver of using a kidney donor having the SCT are still unknown. The screening of potential kidney donors for SCT has to be promoted. The current general view is that agreement for a living SCT graft donor transplantation should be carefully evaluated. Very few data are available in SCT recipients. One-year graft and patient survival are 63% and 83% in a short series of SCT recipients (N = 21) (Chatterjee et al., 1980). Of note, 10% of patients experienced painful crisis after transplantation.

The hypothesis that SCT may be a risk factor for early renal allograft loss requires further evaluation.

Table 167.3 Renal pathologic findings in sickle cell trait (prevalence is unknown)

Renal features	Comments	References
Urinary concentration impairment	Obligatory loss of free water: favours exercise-related complications	Gupta et al., 1991
Haematuria	Most frequent complication (related to papillary necrosis in most cases)	Kiryluk et al., 2007
Papillary necrosis	Predominantly affecting left kidney	Eckert et al., 1974
Albuminuria	Increased risk among type 2 diabetic patients and polycystic kidney disease patients.	Ajayi and Kolawole, 2004
Acute kidney injury	Associated with rhabdomyolysis	Tsaras et al., 2009
Chronic renal failure	Controversial	Derebail et al., 2010; Hicks et al., 2011
Renal medullary carcinoma	Very rare in patients without HbS 3 time more frequent in right kidney 120 cases described	Watanabe et al., 2007
Asymptomatic bacteriuria in pregnancy	Likely association	Whalley et al., 1964
Renal allograft failure	Renal vein thrombosis (3 case reports) 35% graft loss at 1 year	Chatterjee, 1980; Kim et al., 2011

Conclusion

The substantial improvement in survival of homozygous sickle cell patients through specific medical programmes has raised new medical concerns such as SCAN. This nephropathy is suspected by a hyperfiltration with or without albuminuria and may lead to CKD progression and ultimately to ESRD. Indeed, several additional factors such as papillary necrosis (due to vascular thrombosis), nephrotoxic drugs, and/or infections may further be deleterious with a special emphasis on blood hypertension which is underestimated (relative hypertension). In most cases, renal biopsy is thus unnecessary. Clinical trials aiming for nephroprotection are mandatory in patients with stage 1 CKD in order to assess the usefulness of drugs such as ACEIs. General guidelines (Lionnet et al., 2009) should be promoted in order to define specific recommendation and biological targets.

References

Abbott, K. C., Hypolite, I. O., and Agodoa, L. Y. (2002). Sickle cell nephropathy at end-stage renal disease in the United States: patient characteristics and survival. *Clin Nephrol*, 58, 9–15.

Ajayi, A. A. and Kolawole, B. A. (2004). Sickle cell trait and gender influence type 2 diabetic complications in African patients. *Eur J Intern Med*, 15, 312–5.

Allon, M. (1990). Renal abnormalities in sickle cell disease. *Arch Intern Med*, 150, 501–4.

Alvarez, O., Lopez-Mitnik, G., and Zilleruelo, G. (2008). Short-term follow-up of patients with sickle cell disease and albuminuria. *Pediatr Blood Cancer*, 50, 1236–9.

Alvarez, O., Miller, S. T., Wang, W. C., *et al.* (2012). Effect of hydroxyurea treatment on renal function parameters: results from the multi-center placebo-controlled baby hug clinical trial for infants with sickle cell anemia. *Pediatr Blood Cancer*, 59, 668–74.

Alvarez, O., Montane, B., Lopez, G., *et al.* (2006). Early blood transfusions protect against microalbuminuria in children with sickle cell disease. *Pediatr Blood Cancer*, 47, 71–7.

Anderson, S. and Vora, J. P. (1995). Current concepts of renal hemodynamics in diabetes. *J Diabetes Complications*, 9, 304–7.

Aoki, R. Y. and Saad, S. T. (1995). Enalapril reduces the albuminuria of patients with sickle cell disease. *Am J Med*, 98, 432–5.

Ashcroft, M. T. and Desai, P. (1976). Mortality and morbidity in Jamaican adults with sickle cell trait and with normal hemoglobin followed up for 12 years. *Lancet*, 2, 784–6.

Asnani, M. R., Fraser, R. A., and Reid, M. E. (2011). Higher rates of hemolysis are not associated with albuminuria in Jamaicans with sickle cell disease. *PLoS One*, 6, e18863.

Audard, V., Homs, S., Habibi, A., *et al.* (2010). Acute kidney injury in sickle patients with painful crisis or acute chest syndrome and its relation to pulmonary hypertension. *Nephrol Dial Transplant*, 25, 2524–9.

Aygun, B., Mortier, N. A., Smeltzer, M. P., *et al.* (2011). Glomerular hyperfiltration and albuminuria in children with sickle cell anemia. *Pediatr Nephrol*, 26, 1285–90.

Aygun, B., Mortier, N. A., Smeltzer, M. P., *et al.* (2013). Hydroxyurea treatment decreases glomerular hyperfiltration in children with sickle cell anemia. *Am J Hematol*, 88,116–19.

Bains, S. K., Foresti, R., Howard, J., *et al.* (2010). Human sickle cell blood modulates endothelial heme oxygenase activity: effects on vascular adhesion and reactivity. *Arterioscler Thromb Vasc Biol*, 30(2), 305–12.

Bakir, A. A., Hathiwala, S. C., Ainis, H., *et al.* (1987). Prognosis of the nephrotic syndrome in sickle glomerulopathy. A retrospective study. *Am J Nephrol*, 7, 110–15.

Balla, J., Vercellotti, G. M., Jeney, V., *et al.* (2007). Heme, heme oxygenase, and ferritin: how the vascular endothelium survives (and dies) in an iron-rich environment. *Antioxid Redox Signal*, 9, 2119–37.

Bank, N. Aynedjian, H. S., Qiu, J. H., *et al.* (1996). Renal nitric oxide synthases in transgenic sickle cell mice. *Kidney Int*, 50, 184–9.

Barros, F. B., Lima, C. S., Santos, A. O., *et al.* (2006). 51Cr-EDTA measurements of the glomerular filtration rate in patients with sickle cell anaemia and minor renal damage. *Nucl Med Commun*, 27, 959–62.

Becton, L. J., Kalpatthi, R. V., Rackoff, E., *et al.* (2010). Prevalence and clinical correlates of microalbuminuria in children with sickle cell disease. *Pediatr Nephrol*, 25, 1505–11.

Berg, U. B. (2006). Differences in decline in GFR with age between males and females. Reference data on clearances of inulin and PAH in potential kidney donors. *Nephrol Dial Transplant*, 21, 2577–8.

Bernstein, J. and Whitten, C. F. (1960). A histologic appraisal of the kidney in sickle cell anemia. *Arch Pathol*, 70, 407–18.

Bhathena, D. B. and Sondheimer, J. H. (1991). The glomerulopathy of homozygous sickle hemoglobin (SS) disease: morphology and pathogenesis. *J Am Soc Nephrol*, 1, 1241–52.

Bolarinwa, R. A., Akinlade, K. S., Kuti, M. A., *et al.* (2012). Renal disease in adult Nigerians with sickle cell anemia: a report of prevalence, clinical features and risk factors. *Saudi J Kidney Dis Transpl*, 23, 171–5.

Bonnin, P., Sabaa, N., Flamant, M., *et al.* (2008). Ultrasound imaging of renal vaso-occlusive events in transgenic sickle mice exposed to hypoxic stress *Ultrasound Med Biol*, 34, 1076–84.

Botros, F. T. and Navar, L. G. (2006). Interaction between endogenously produced carbon monoxide and nitric oxide in regulation of renal afferent arterioles. *Am J Physiol Heart Circ Physiol*, 291, H2772–8.

Botros, F. T., Prieto-Carrasquero, M. C., Martin, V. L., *et al.* (2008). Heme oxygenase induction attenuates afferent arteriolar autoregulatory responses. *Am J Physiol Renal Physiol*, 295, F904–11.

Botros, F. T., Schwartzman, M. L., Stier, C. T., Jr., *et al.* (2005). Increase in heme oxygenase-1 levels ameliorates renovascular hypertension. *Kidney Int*, 68, 2745–55.

Bradley, S. E. (1947). Renal function during chronic anemia in man. *Blood*, 2, 192–202.

Brenner, B. M., Meyer, T. W., and Hostetter, T. H. (1982). Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med*, 307, 652–9.

Buckalew, V. M. and Someren, A. (1974). Renal manifestations of sickle cell disease. *Arch Intern Med*, 133, 660–9.

Chatterjee, S. N. (1980). National study on natural history of renal allografts in sickle cell disease or trait, *Nephron*, 25(4), 199–201.

Chiarelli, F., Cipollone, F., Romano, F., *et al.* (2000). Increased circulating nitric oxide in young patients with type 1 diabetes and persistent microalbuminuria: relation to glomerular hyperfiltration. *Diabetes*, 49, 1258–63.

Dasgupta, T., Fabry, M. E., and Kaul, D. K. (2010). Antisickling property of fetal hemoglobin enhances nitric oxide bioavailability and ameliorates organ oxidative stress in transgenic-knockout sickle mice. *Am J Physiol Regul Integr Comp Physiol*, 298, R394–402.

Davis, C. J., Mostofi, F. K., and Sestarhenn, I. A. (1995). Renal medullary carcinoma. The seventh sickle cell nephropathy. *Am J Surg Pathol*, 19, 1–11.

Day, T. G., Drasar, E., Fulford, T., *et al.* (2012). Association between hemolysis and albuminuria in adults with sickle cell anemia. *Haematologica*, 97, 201–5.

DeFronzo de R. A., Taufeld, PA, Black H, McPhedran, P, Cooke CR, (1979). Impaired renal tubular potassium secretion in sickle cell disease. *Ann Int Med*, 90, 310–16.

De Franceschi, L., Brugnara, C., Rouyer-Fessard, P., *et al.* (1999). Formation of dense erythrocytes in SAD mice exposed to chronic hypoxia: evaluation of different therapeutic regimens and of a combination of oral clotrimazole and magnesium therapies. *Blood*, 94, 4307–13.

De Jong, P. E., de Jong-van Den Berg, L. T., Statius van Eps, L. W. (1978) The tubular reabsorption of phosphate in sickle-cell nephropathy. *Clin Sci Mol Med*, 55, 429–34.

De Jong, P. E. and Statius van Eps, L. W. (1985). Sickle cell nephropathy: new insights into its pathophysiology. *Kidney Int*, 27, 711–7.

De Paepe, M. E. and Trudel, M. (1994). The transgenic SAD mouse: a model of human sickle cell glomerulopathy. *Kidney Int*, 46, 1337–45.

Derebail, V. K., Nachman, P. H., Key, N. S., *et al.* (2010). High prevalence of sickle cell trait in African Americans with ESRD. *J Am Soc Nephrol*, 21, 413–7.

Devereux, S. and Knowles, S. M. (1985). Rhabdomyolysis and acute renal failure in sickle cell anaemia. *Br Med J*, 290, 1707.

Dharnidharka, V. A., Dabbagh, S., Atiyeh, B., *et al.* (1998). Prevalence of microalbuminuria in children with sickle cell disease. *Pediatr Nephrol*, 12, 475–8.

Donnellv, P. K., Edmunds, M. E., and O'Reilly, K. (1988). Renal transplantation in sickle cell disease. *Lancet*, 110, 229.

Eberhardt, R. T., McMahon, L., Duffy, S. J., *et al.* (2003). Sickle cell anemia is associated with reduced nitric oxide bioactivity in peripheral conduit and resistance vessels. *Am J Hematol*, 74, 104–11.

Eckert, D. E., Jonitlis, A. J., and Davidson, A. J. (1974). The incidence and manifestation of urographic papillary abnormalities in patients with S hemoglobinopathies. *Radiology*, 113, 59–63.

Elfenbein, I. B., Patchefsky, A., Schwartz, W., *et al.* Pathology of the glomerulus in sickle cell anemia with and without nephrotic syndrome. *Am J Pathol*, 77, 357–74.

Embury, S. H., Dozy, A. M., Miller, J., *et al.* (1982) Concurrent sickle-cell anemia and alpha-thalassemia: effect on severity of anemia. *N Engl J Med*, 306, 270–4.

Etteldorf, J. N., Smith, J. D., Tuttle, A. H., *et al.* (1955). Renal hemodynamic studies in adults with sickle cell anemia. *Am J Med*, 18, 243–8.

Etteldorf, J. N., Tuttle, A. W., and Clayton, G. W. (1952). Renal function studies in pediatrics. Renal hemodynamics in children with sickle cell anemia. *Am J Dis Child*, 83, 185–91.

Fabry, M. E., Sengupta, A., Suzuka, S. M., *et al.* (1995). A second generation transgenic mouse model expressing both hemoglobin S (HbS) and HbS-Antilles results in increased phenotypic severity. *Blood*, 86, 2419–28.

Falk, R. J. and Jeannette, J. C. (1994). Sickle cell nephropathy. *Adv Nephrol*, 23, 133–47.

Falk, R. J., Scheinman, J., Phillips, G., *et al.* (1992). Prevalence and pathologic features of sickle cell nephropathy and response to inhibition of angiotensin-converting enzyme. *N Engl J Med*, 326, 910–5.

Fitzhugh, C. D., Wigfall, D. R., and Ware, R. E. (2005). Enalapril and hydroxyurea therapy for children with sickle nephropathy. *Pediatr Blood Cancer*, 45, 982–5.

Foucan, L., Bourhis, V., Bangou, J., *et al.* (1998). A randomized trial of captopril for microalbuminuria in normotensive adults with sickle cell anemia. *Am J Med*, 104, 339–42.

Gladwin, M. T. and Kato, G. J. (2005). Cardiopulmonary complications of sickle cell disease: role of nitric oxide and hemolytic anemia. *Hematology Am Soc Hematol Educ Program*, 2005, 51–7.

Gladwin, M. T., Lancaster, J. R., Jr., Freeman, B. A., *et al.* (2003). Nitric oxide's reactions with hemoglobin: a view through the SNO-storm. *Nat Med*, 9, 496–500.

Gordeuk, V. R., Sachdev, V., Taylor, J. G., *et al.* (2008). Relative systemic hypertension in patients with sickle cell disease is associated with risk of pulmonary hypertension and renal insufficiency. *Am J Hematol*, 83, 15–18.

Graido-Gonzalez, E., Doherty, J. C., Bergreen, E. W., *et al.* (1998). Plasma endothelin-1, cytokine, and prostaglandin E2 levels in sickle cell disease and acute vaso-occlusive sickle crisis. *Blood*, 92, 2551–5.

Guasch, A., Cua, M., You, W., *et al.* (1997). Sickle cell anemia causes a distinct pattern of glomerular dysfunction. *Kidney Int*, 51(3), 826–33.

Guasch, A., Navarrete, J., Nass, K., *et al.* (2006). Glomerular involvement in adults with sickle cell hemoglobinopathies. Prevalence and clinical correlates of progressive renal failure. *J Am Soc Nephrol*, 17, 2228–35.

Gupta, A. K., Kirchner, K. A., Nicholson, R., *et al.* (1991). Effects of alpha thalassemia and sickle polymerization tendency on the urine-concentrating defects of individuals with sickle cell trait. *J Clin Invest*, 88, 1963–8.

Gurkan, S., Scarponi, K. J., Hotchkiss, H., *et al.* (2010). Lactate dehydrogenase as a predictor of kidney involvement in patients with sickle cell anemia. *Pediatr Nephrol*, 25, 2123–7.

Hammerman, S. I., Kourembanas, S., Conca, T. J., *et al.* (1997). Endothelin-1 production during the acute chest syndrome in sickle cell disease. *Am J Respir Crit Care Med*, 156, 280–5.

Hassell, K. L., Eckman, J. R., and Lane, P. A. (1994). Acute multiorgan failure syndrome: a potentially catastrophic complication of severe sickle cell pain episodes. *Am J Med*, 96, 155–62.

Hatch, F. E. Jr., Azar, S. H., Ainsworth, T. E., *et al.* (1970). Renal circulatory studies in young adults with sickle cell anemia. *J Lab Clin Med*, 76(4), 632–40.

Hatch, F. E., Crowe, L. R., Miles, D. E., *et al.* (1989). Altered vascular reactivity in sickle hemoglobinopathy. A possible protective factor from hypertension. *Am J Hypertens*, 2, 2–8.

Hatch, F. E., Culbertson, J. W., and Diggs, L. W. (1967). Nature of the renal concentrating defect in sickle cell disease. *J Clin Invest*, 46, 336–45.
Haymann, J. P., Stankovic, K., Levy, P., *et al.* (2010). Glomerular hyperfiltration in adult sickle cell anemia: a frequent hemolysis associated feature. *Clin J Am Soc Nephrol*, 5, 756–61.
Herrera, J., Avila, E., Marín, C., *et al.* (2002). Impaired creatinine secretion after an intravenous creatinine load is an early characteristic of the nephropathy of sickle cell anaemia. *Nephrol Dial Transplant*, 17, 602–7.
Herrick, J. B. (1910). Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia. *Arch Intern Med*, 6, 517–21.
Hicks, P. J., Langefeld, C. D., Lu, L., *et al.* (2011). Sickle cell trait is not independently associated with susceptibility to end-stage renal disease in African Americans. *Kidney Int*, 80, 1339–43.
Higgs, D. R., Aldridge, B. E., Lamb, J., *et al.* (1982). The interaction of alpha-thalassemia and homozygous sickle-cell disease. *N Engl J Med*, 306, 1441–6.
Hostetter, T. H., Olson, J. L., Rennke, H. G., *et al.* (1982). Altered glomerular permselectivity and progressive sclerosis following extreme ablation of renal mass. *Kidney Int*, 22, 112–26.
Hsu, L. L., Champion, H. C., Campbell-Lee, S. A., *et al.* (2007). Hemolysis in sickle cell mice causes pulmonary hypertension due to global impairment in nitric oxide bioavailability. *Blood*, 109, 3088–98.
Huang, E., Parke, C., Mehrnia, A., *et al.* (2013). Improved survival among sickle cell kidney transplant recipients in the recent era. *Nephrol Dial Transplant*, 28(4), 1039–46.
Ichihara, A., Inscho, E. W., Imig, J. D., *et al.* (1998). Neuronal nitric oxide synthase modulates rat renal microvascular function. *Am J Physiol*, 274, F516–24.
Juncos, J. P., Grande, J. P., Croatt, A. J., *et al.* (2010). Early and prominent alterations in hemodynamics, signaling, and gene expression following renal ischemia in sickle cell disease. *Am J Physiol Renal Physiol*, 298, F892–9.
Kato, G. J., McGowan, V., Machado, R. F., *et al.* (2006). Lactate deshydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. *Blood*, 107, 2279–85.
Kaul, D. K. and Fabry, M. E. (2004). In vivo studies of sickle red blood cells. *Microcirculation*, 11, 153–65.
Kaul, D. K., Liu, X. D., Fabry, M. E., *et al.* (2000). Impaired nitric oxide-mediated vasodilation in transgenic sickle mouse. *Am J Physiol Heart Circ Physiol*, 278, H1799–806.
Kelly, C. J. and Singer, I. (1986). Acute renal failure in sickle-cell disease. *Am J Kidney Dis*, 8, 146–50.
Kharitonov, V. G., Sharma, V. S., Pilz, R. B., *et al.* (1995). Basis of guanylate cyclase activation by carbon monoxide. *Proc Natl Acad Sci U S A*, 92, 2568–71.
Kim, L., Garfinkel, M. R., Chang, A., *et al.* (2011). Intragraft vascular occlusive sickle crisis with early renal allograft loss in occult sickle cell trait. *Hum Pathol*, 42, 1027–33.
King, L., MooSang, M., Miller, M., *et al.* (2011). Prevalence and predictors of microalbuminuria in Jamaican children with sickle cell disease. *Arch Dis Child*, 96, 1135–9.
Kiryluk, K., Jadron, A., Gupta, M., *et al.* (2007). Sickle cell trait and gross hematuria. *Kidney Int*, 71, 706–10.
Leight, L., Snider, T. H., Clifford, G. O., *et al.* (1954). Hemodynamic studies in sickle cell anemia. *Circulation*, 10, 653–62.
Levitt, M. F., Hauser, A. D., Levy, M. S., *et al.* (1960). The renal concentrating defect in sickle cell disease. *Am J Med*, 29, 611–22.
Lionnet, F., Arlet, J. B., Bartolucci, P., *et al.* (2009). Recommandations pratiques de prise en charge de la drépanocytose de l'adulte. *Rev Med Interne*, 3, S162–223.
Lionnet, F., Hammoudi, N., Stankovic Stojanovic, K., *et al.* (2012). HbSC disease complications: a clinical study of 179 cases. *Haematologica*, 97, 1136–41.
Liu, Q., Galli, S., Srinivasan, R., *et al.* (2013). Renal medullary carcinoma: molecular, immunohistochemistry, and morphologic correlation. *Am J Surg Pathol*, 37, 368–74.
Loutzenhiser, R., Bidani, A., and Chilton, L. (2002). Renal myogenic response: kinetic attributes and physiological role. *Circ Res*, 90, 1316–24.
Maier-Redelsperger, M., Lévy, P., Lionnet, F., *et al.* (2010). Strong association between a new marker of hemolysis and glomerulopathy in sickle cell anemia. *Blood Cells Mol Dis*, 45, 289–92.
Maigne, G., Ferlicot, S., Galacteros, F., *et al.* (2010). Glomerular lesions in patients with sickle cell disease. *Medicine*, 89, 18–27.
Manci, E., Culberson, D. E., Yang, Y. M., *et al.* (2003). Causes of death in sickle cell disease: an autopsy study. *Br J Haematol*, 123, 359–65.
Marouf, R., Mojiminiyi, O., Abdella, N., *et al.* (2006). Comparison of renal function markers in Kuwaiti patients with sickle cell disease. *J Clin Pathol*, 59, 345–51.
McBurney, P. G., Hanevold, C. D., Hernandez, C. M., *et al.* (2002). Risk factors for microalbuminuria in children with sickle cell anemia. *J Pediatr Hematol Oncol*, 24, 474–7
McCall, I. W., Moule, N., Desai, P., *et al.* (1978). Urographic findings in homozygous sickle cell disease. *Radiology*, 126, 99–104.
McCoy, R. C. (1969). Ultrastructural alterations in the kidney of patients with sickle cell disease and the nephrotic syndrome. *Lab Invest*, 21, 85–95.
McKie, K. T., Hanevold, C. D., Hernandez, C., *et al.* (2007). Prevalence, prevention, and treatment of microalbuminuria and proteinuria in children with sickle cell disease. *J Pediatr Hematol Oncol*, 29, 140–4.
McPherson Yee, M., Jabbar, S. F., Osunkwo, I., *et al.* (2011). Chronic kidney disease and albuminuria in children with sickle cell disease. *Clin J Am Soc Nephrol*, 6, 2628–33.
Montgomery, R., Zibari, G., Hill, G. S., *et al.* (1994). Renal transplantation in patients with sickle cell nephropathy. *Transplantation*, 58, 618–20.
Morgan, A. G., de Ceuler, K., and Serjeant, G. R. (1984). Glomerular function and hypericaemia in sickle cell disease. *J Clin Path*, 37, 1046–9.
Morris, C. R., Kato, G. J., Poljakovic, M., *et al.* (2005). Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA*, 294, 81–90.
Mozzarelli, A., Hofrichter, J., and Eaton, W. A. (1987). Delay time of hemoglobin S polymerization prevents most cells from sickling in vivo. *Science*, 237, 500–6.
Nasr, S. H., Markowitz, G. S., Sentman, R. L., *et al.* (2006). Sickle cell disease, nephrotic syndrome, and renal failure. *Kidney Int*, 69, 1276–80.
Nath, K. A., Grande, J. P., Croatt, A. J., *et al.* (2005). Transgenic sickle mice are markedly sensitive to renal ischemia-reperfusion injury. *Am J Pathol*, 166(4), 963–72.
Nath, K. A., Hernandez, M. C., Croatt, A. J., *et al.* (2010). Heme oxygenase activity as a determinant of the renal hemodynamic response to low-dose ANG II. *Am J Physiol Regul Integr Comp Physiol*, 299(5), R1183–91.
Nath, K. A. and Katusic, Z. S. (2012). Vasculature and kidney complications in sickle cell disease. *J Am Soc Nephrol* 23, 781–784.
Nath, K. A., Katusic, Z. S., and Gladwin, M. T. (2004). The perfusion paradox and vascular instability in sickle cell disease. *Microcirculation*, 11, 179–93.
Nath, K. A., Shah, V., Haggard, J. J., *et al.* (2000). Mechanisms of vascular instability in a transgenic mouse model of sickle cell disease. *Am J Physiol Regul Integr Comp Physiol*, 279, R1949–55.
Nebor, D., Broquere, C., Brudey, K., *et al.* (2010). Alpha-thalassemia is associated with a decreased occurrence and a delayed age-at-onset of albuminuria in sickle cell anemia patients. *Blood Cells Mol Dis*, 45, 154–8.
Odita, J. C., Ugbodaga, C. I., Okafor, L. A., *et al.* (1983). Urographic findings in homozygous sickle cell disease. *Diagn Imaging*, 52, 259–63.
Ojo, A. O., Govaerts, T. C., Schmouder, R. L., *et al.* (1999). Renal transplantation in end-stage sickle cell nephropathy. *Transplantation*, 67, 291–5.
Osterby, R. (1992). Glomerular structural changes in type 1 (insulin-dependent) diabetes mellitus: causes, consequences, and prevention. *Diabetologia*, 35, 803–12.
Pallone, T. L. (2007). Control of renal Na+ excretion by heme oxygenase. *Hypertension*, 49(1), 23–4.

Pardo, V., Strauss, J., Kramer, H., *et al.* (1975). Nephropathy associated with sickle cell anemia: an autologous immune complex nephritis ii. clinicopathologic study of seven patients. *Am J Med*, 59, 650–9.
Pászty, C., Brion, C. M., Manci, E., *et al.* (1997). Transgenic knockout mice with exclusively human sickle hemoglobin and sickle cell disease. *Science*, 278, 876–8.
Pegelow, C. H., Colangelo, L., Steinberg, M., *et al.* (1997). Natural history of blood pressure in sickle cell disease: risks for stroke and death associated with relative hypertension in sickle cell anemia. *Am J Med*, 102, 171–7.
Pitcock, J. A., Muirhead, E. E., Hatch, F. E., *et al.* (1970). Early renal changes in sickle cell anemia. *Arch Pathol*, 90, 403–10.
Platt, O. S., Brambilla, D. J., Rosse, W. F., *et al.* (1994). Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N Engl J Med*, 330, 1639–44.
Powars, D. R., Chan, L. S., Hiti, A., *et al.* (2005). Outcome of sickle cell anemia: a 4-decade observational study of 1056 patients. *Medicine (Baltimore)*, 84, 363–76.
Powars, D. R., Elliot-Mills, D. D., Chan, L., *et al.* (1991). Chronic renal failure in sickle cell disease: risk factors, clinical course, and mortality. *Ann Intern Med*, 115, 614–20.
Prasannan, L., Flynn, J. T., and Levine, J. E. (2003). Acute renal failure following deferoxamine overdose. *Pediatr Nephrol*, 18, 283–5.
Quek, L., Sharpe, C., Dutt, N., *et al.* (2010). Acute human parvovirus B19 infection and nephrotic syndrome in patients with sickle cell disease. *Br J Haematol*, 149, 289–91.
Reboldi, G., Gentile, G., Angeli, F., *et al.* (2009). Choice of ACE inhibitor combinations in hypertensive patients with type 2 diabetes: update after recent clinical trials. *Vasc Health Risk Manag*, 5, 411–27.
Reiter, C. D., Wang, X. D., Tanus-Santos, J. E., *et al.* (2002). Cell-free hemoglobin limits nitric oxide bioavailability in sickle cell disease. *Nat Med*, 8, 1383–9.
Rees, D. C., Williams, T. N., and Gladwin, M. T. (2010). Sickle-cell disease. *Lancet*, 376, 2018–31.
Rodgers, G. P., Walker, E. C., and Podgor, M. J. (1993). Is 'relative' hypertension a risk factor for vaso-occlusive complications in sickle cell disease? *Am J Med Sci*, 305, 150–6.
Rother, R. P., Bell, L., Hillmen, P., *et al.* (2005). The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin. *JAMA*, 293, 1653–62.
Roy, S. B., Bhatia, M. L., Mathur, V. S., *et al.* (1963). Hemodynamic effects of chronic severe anemia. *Circulation*, 28, 346–56.
Rybicki, A. C. and Benjamin, L. J. (1998). Increased levels of endothelin-1 in plasma of sickle cell anemia patients. *Blood*, 92, 2594–6.
Ryan, T. M., Ciavatta, D. J., and Townes, T. M. (1997). Knockout-transgenic mouse model of sickle cell disease. *Science*, 278(5339), 873–6.
Sabaa, N., de Franceschi, L., Bonnin, P., *et al.* (2008). Endothelin receptor antagonism prevents hypoxia-induced mortality and morbidity in a mouse model of sickle cell disease. *J Clin Invest*, 118, 1924–33.
Saborio, P. and Scheinman, J. I. (1999). Sickle cell nephropathy. *J Am Soc Nephrol*, 10, 187–92.
Schaller, S. and Kaplan, B. S. (1998). Acute nonoliguric renal failure in children associated with nonsteroidal antiinflammatory agents. *Pediatr Emerg Care*, 14, 416–8.
Scheinman, J. I. (2009). Sickle cell disease and the kidney. *Nat Clin Pract Nephrol*, 5(2), 78–88.
Schmitt, F., Martinez, F., Brillet, G., *et al.* (1998). Early glomerular dysfunction in patients with sickle cell anemia. *Am J Kidney Dis*, 32, 208–14.
Schwartz, G. J., Brion, L. P., and Spitzer, A. (1987). The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children and adolescents. *Pediatr Clin North Am*, 34, 571–90.
Serjeant, G. R., Higgs, D. R., and Hambleton, I. R. (2007). Elderly survivors with homozygous sickle cell disease. *N Engl J Med*, 356, 642–3.
Serjeant, G. R., Serjeant, B. E., Mason, K. P., *et al.* (2009). The changing face of homozygous sickle cell disease: 102 patients over 60 years. *Int J Lab Hematol*, 31, 585–96.
Sharpe, C. C. and Thein, S. L. (2011). Sickle cell nephropathy—a practical approach. *Br J Haematol*, 155, 287–97.
Sklar, A. H., Campbell, H., Caruana, R. J., *et al.* (1990). A population study of renal function in sickle cell anemia. *Int J Artif Organs*, 13, 231–6.
Sochett, E. B., Cherney, D. Z., Curtis, J. R., *et al.* (2006). Impact of renin angiotensin system modulation on the hyperfiltration state in type 1 diabetes. *J Am Soc Nephrol*, 17, 1703–9.
Stallworth, J. R., Tripathi, A., and Jerrell, J. M. (2011). Prevalence, treatment, and outcomes of renal conditions in pediatric sickle cell disease. *South Med J*, 104, 752–6.
Statius van Eps, L. W., Pinedo-Veels, C., De Vries, G. H., *et al.* (1970). Nature of concentrating defect in sickle cell nephropathy. *Lancet*, 1, 450–52.
Statius van Eps, L. W., Schouten, H., La Porte-Wijsman, L. W., *et al.* (1967). The influence of red blood cell transfusions on the hyposthenuria and renal hemodynamics of sickle cell anemia. *Clin Chim Acta*, 17, 449–61.
Takenaka, T., Harrison-Bernard, L. M., Inscho, E. W., *et al.* (1994). Autoregulation of afferent arteriolar blood flow in juxtamedullary nephrons. *Am J Physiol*, 267, F879–87.
Tejani, A., Phadke, K., Adamson, O., *et al.* (1985). Renal lesions in sickle cell nephropathy in children. *Nephron*, 39, 352–5.
Tharaux, P. L., Hagège, I., Placier, S., *et al.* (2005). Urinary endothelin-1 as a marker of renal damage in sickle cell disease. *Nephrol Dial Transplant*, 20, 2408–13.
Thomson, J., Reid, M., Hambleton, I., *et al.* (2007). Albuminuria and renal function in homozygous sickle cell disease observations from a cohort study. *Arch Intern Med*, 167, 701–8.
Trudel, M., Saadane, N., Garel, M. C., *et al.* (1991). Towards a transgenic mouse model of sickle cell disease: hemoglobin SAD. *EMBO J*, 10, 3157–65.
Tsaras, G., Owusu-Ansah, A., Owusua Boateng, F., *et al.* (2009). Complications associated with sickle cell trait: a brief narrative review. *Am J Med*, 122, 507–12.
Vaamonde, C. A. (1984). Renal papillary necrosis in sickle cell hemoglobinopathies. *Semin Nephrol*, 4, 48–64.
Van Deventer, H. E., George, J. A., Paiker, J. E., *et al.* (2008). Estimating glomerular filtration rate in black South Africans by use of the modification of diet in renal disease and Cockroft-Gault equation. *Clin Chem*, 54, 1197–202.
Vedel, P., Obel, J., Nielsen, F. S., Svendsen, T. L., *et al.* (1996). Glomerular hyperfiltration in microalbulminuric NIDDM patients. *Diabetologia*, 39, 1584–9.
Villers, M. S., Jamison, M. G., De Castro, L. M., *et al.* (2008). Morbidity associated with sickle cell disease in pregnancy. *Am J Obstet Gynecol*, 199, 125.e1–5.
Wang, G., Hamid, T., Keith, R. J., *et al.* (2010). Cardioprotective and antiapoptotic effects of heme oxygenase-1 in the failing heart. *Circulation*, 121, 1912–25.
Warady, B. A. and Sullivan, E. K. (1998). Renal transplantation in children with sickle cell disease: a report of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS). *Pediatr Transplant*, 2, 130–3.
Ware, R. E., Rees, R. C., Sarnaik, S. A., *et al.* (2010). Renal function in infants with sickle cell anemia: baseline data from the BABY HUG trial. *J Pediatr*, 156, 66–70.
Watanabe, I. C., Billis, A., Guimares, M. S., *et al.* (2007). Renal medullary carcinoma: report of seven cases from Brazil. *Mod Pathol*, 20, 914–20.
Weatherall, D. J. (2010). The inherited diseases of hemoglobin are an emerging global health burden. *Blood*, 115, 4331–6.
Whalley, P. J., Martin, F. G., and Pritchard, J. A. (1964). Sickle cell trait and urinary tract infection during pregnancy. *JAMA*, 189, 903–6.
Wierenga, K. J., Hambleton, I. R., and Lewis, N. A. (2001). Survival estimates for patients with homozygous sickle-cell disease in Jamaica: a clinic-based population study. *Lancet*, 3, 357, 680–3.
Wierenga, K. J., Pattison, J. R., Brink, N., *et al.* (1995). Glomerulonephritis after human parvovirus infection in homozygous sickle-cell disease. *Lancet*, 346, 475–6.
Wigfall, D. R., Ware, R. E., Burchinal, M. R., *et al.* (2000). Prevalence and clinical correlates of glomerulopathy in children with sickle cell disease. *J Pediatr*, 136, 749–53.

CHAPTER 168

The obese patient (metabolic syndrome)

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Introduction

In chronic kidney disease (CKD), the intermediary metabolism and the endocrine and hormonal systems undergo profound changes, contributing to alterations in most organ systems as well as nutritional status, including body composition. Most of these *metabolic* and *nutritional changes in CKD*, such as insulin resistance, lipid abnormalities, protein-energy wasting (PEW), and cardiovascular disease (CVD), become progressively worse as CKD progresses into end-stage renal disease (ESRD). Although many alterations may improve with the initiation of dialysis treatment, other aspects are not affected, or may even deteriorate in dialysis patients, mainly as a consequence of the partial or total loss of the residual renal function, but also due to untoward effects of the dialysis treatment. Kidney transplantation usually results in major improvements of health and quality of life but may also result in increased incidence of obesity and metabolic syndrome (MetS), mainly due to the immunosuppressive treatment with corticosteroids and other drugs. On the other hand, the presence of nutritional abnormalities, such as MetS and obesity and related comorbidities, such as diabetes and CVD, play an important role in the initiation and progression of CKD.

Aetiology

Overweight and *obesity* are global public health problems in themselves and because of their roles as underlying causes of several major diseases: diabetes mellitus, sleep apnoea, cardio- and cerebrovascular disease, musculoskeletal and psychosocial disorders, and some types of cancer (World Health Organization, 2011). In particular, abdominal obesity, one of the main components of MetS, is thought to play a detrimental role (Box 168.1) (4S Study Group, 1994; Arner, 2003).

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health (World Health Organization, 2011) and are the result of an imbalance between energy intake and expenditure. The definition is based on body mass index (BMI), and is widely used even among CKD patients (Table 168.1). Despite the limitations of BMI, especially that it makes no distinction between fat and lean body, this index is easily applied, internationally recognized, and widely used as a measure of obesity even among CKD patients. Factors as age, gender, ethnicity, fluid balance, muscle depletion-related PEW, exercise, various diseases, and nutritional intake can confound body weight measures in CKD patients (Fouque et al., 2008). Combining measurement of weight with an assessment of body composition is recommended (Table 168.2). The study of longitudinal changes in body composition in CKD patients is important as *obese sarcopenia*, which is defined as relative PEW and loss of lean body mass (LBM), is not an uncommon finding among overweight patients and is often associated with inflammation and increased incidence CVD in CKD patients (Honda et al., 2007). Indirect measures of body composition (waist:hip ratio, sagittal abdominal diameter, and the conicity index) can be used as indirect measures of body composition in CKD patients. They correlate with the amount of visceral fat mass (Velludo et al., 2008) reflecting *abdominal* or *central obesity* (Björntorp, 1992; de Koning et al., 2007) which in ESRD predicts outcome better than BMI (Elsayed et al., 2008; Cordeiro et al., 2010; Postorino et al., 2011). The prognostic value of different measures of weight and body composition may vary during different stages of CKD (Kamimura et al., 2003; Barreto Silva et al., 2008). WHR may actually modify the risk of hypertriglyceridemia, leptin, and adiponectin in all-cause and cardiovascular mortality in dialysis patients (Postorino et al., 2011; Zoccali et al., 2011).

Overweight and obesity are associated with a higher risk of CKD (Coresh et al., 2007), both independently and through shared risk factors, such as diabetes and hypertension (Honda et al., 2007). A systematic review and meta-analysis showed strong association between overweight and the risk of kidney disease (Wang et al., 2008) (Fig. 168.1). However, the effect and consequences of overweight and obesity on morbidity and mortality differ markedly throughout CKD stages 1–5. On the one hand, these alterations may cause renal disease or contribute to progression of existing CKD. On the other hand, overweight and obesity may play a protective role at later CKD stages by mechanisms that are not yet fully understood (Table 168.3).

The MetS consists of a cluster of clinical traits with metabolic and haemodynamic alterations, which individually or in combination increase the risk for developing overt diabetes, CKD, and other complications leading to increased cardiovascular morbidity and mortality (Table 168.4). Several components of MetS are common already in earlier stages of CKD, either because they are risk factors for developing CKD or because they occur as consequences of CKD. As progression of kidney disease results in further derangements of these metabolic and haemodynamic derangements, advanced stages of CKD and MetS share multiple similarities and multiple interactions. Among obese CKD patients, the uraemic milieu may increase the metabolic consequences of obesity-induced MetS by aggravating

Box 168.1 Risk factors for obesity

- Increased energy intake
- Low physical activity (in relation to energy intake)
- Genetics and epigenetics
- High intake of fat and carbohydrates
- Insulin resistance
- Comorbidity (such as type 2 diabetes mellitus)
- Drugs (such as steroids and insulin).

Table 168.1 International classification of adult non-Asian[a] underweight, overweight, and obesity by body mass index (BMI) according to the World Health Organization

Category	BMI (kg/m^2)
Underweight	< 18.5
Normal weight	18.5–24.9
Overweight	25.0–29.9
Obese	≥ 30
Obese class I	30.0–34.9
Obese class II	35.0–39.9
Obese class III	≥ 40

[a]Other values apply to the Asian population.

inflammatory and oxidative stress pathways (Table 168.4). A recent systematic review and meta-analysis based on > 30,000 patients showed that MetS and its components are risk factors for development of microalbuminuria, overt proteinuria, and CKD stages 3–5 without previous renal disease (Thomas et al., 2011).

Epidemiology

Obesity in chronic kidney disease

Obesity is a risk factor both for linear decline of glomerular filtration rate (GFR) and consequent risk for developing CKD in patients with CKD stages 1–2 (Fox et al., 2004; Gelber et al., 2005; Ejerblad et al., 2006; Hsu et al., 2006; Iseki 2006; Wang et al., 2008). High BMI correlates with lower GFR (cystatin C) in a healthy population with normal estimated GFR and no albuminuria (Muntner et al., 2008). Part of the association between high body weight and decline in GFR may be mediated by hypertension among overweight subjects (Kramer et al., 2005) even after the adjustment for blood pressure (Iseki 2006; Gelber et al., 2007). The risk for developing CKD is markedly increased in subjects with MetS, with or without diabetes (Chen et al., 2004; Kurella et al., 2005; Luk et al., 2008; Park et al., 2010; Lee et al., 2011) and obesity in itself is a negative prognostic factor for further progression of CKD and for morbidity and mortality during CKD stages 1–4 (Table 168.5). On the other hand, loss of LBM occur in the majority of patients with CKD stages 3–5, independent of their initial body weight; this process which is typically combined with a range of other (metabolic, nutritional, hormonal, immunological) alterations including inflammation, is a strong predictor of poor clinical outcome (Heimburger et al., 2000).

Obesity in haemodialysis patients

Whereas low body weight is a poor prognostic sign regardless of dialysis modality (Stack et al., 2004; Ramkumar et al., 2005; Huang et al., 2010), the impact of overweight (BMI > 25 kg/m^2) is unclear and may vary according to the type of dialysis. The inverse relationship between mortality and body weight in haemodialysis (HD) patients is a part of the 'reverse epidemiology' paradox as shown in North American HD patients (Fleischmann et al., 1999; Kalantar-Zadeh et al., 2003, 2005). However, this paradox has not been confirmed in North American non-diabetic patients with mild–moderate CKD (Madero et al., 2007), nor in European HD patients (de Mutsert et al., 2007). A higher prevalence of obesity in the United States, especially in some ethnic groups, as well as dialysis vintage and selection bias—underweight patients have a high mortality—may explain this discrepancy (Table 168.6) (Glanton et al., 2003; Keith et al., 2004). Other fat-related factors potentially contributing to obesity-related survival advantage include better mobilization of endothelial progenitor cells, a lesser uraemic toxin load due to larger distribution volume, a larger prescribed dialysis dose (as overweight resulting in higher volume and therefore a lower Kt/V index, may encourage efforts to increase this index),

Table 168.2 Comparison of methods for estimating body weight and body composition and their prognostic value for predicting morbidity and mortality in CKD

Method	Distinction fat mass/LBM	Easy to use	Cost/benefit	Reproducibility	Prognostic value in CKD
Body mass index	0	+++	+++	+++	+
Waist:hip ratio	0	+++	+++	+++	++
Skinfold thickness	+	++	++	+	++
Bioimpedance	++	+++	++	++	+++
DXA	+++	+	+	+++	+++
Computed tomography	+++	+	+	+++	+++

Ratings (by the authors): +++ excellent; ++ good; + moderate; 0 poor.

CKD = chronic kidney disease; DXA = dual energy X-ray absorptiometry; LBM = lean body mass.

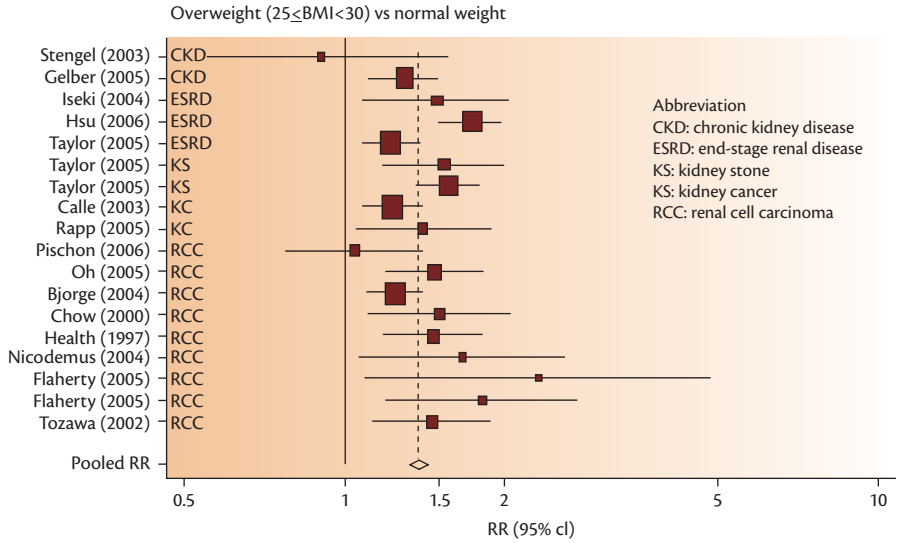

Fig. 168.1 Pooled random-effects estimate of risk ratio and 95% confidence intervals for the association between obesity and kidney disease based on cohort studies in the general populations. Overweight (25 kg/m^2 < BMI < 30 kg/m^2) versus normal weight.
From Wang et al. (2008).

and higher LBM in patients with high BMI. As adipocytes and osteoblasts originate from common progenitor and mesenchymal stem cells it should also be emphasized that obesity is protective against osteoporosis (Table 168.3).

In the short term, increased mortality is seen mainly in patients with PEW and low BMI, as these patients may not survive long enough to eventually suffer from the potential negative long-term effects of obesity, such as dyslipidaemia and enhanced atherogenesis. In general, studies demonstrating 'reverse epidemiology' have rather short follow-up periods, and therefore a potential negative long-term impact of obesity might not be seen. It should be emphasized that obesity has not been shown to have any advantageous effects on self-rated health status, and physical function compared to normal weight or moderately overweight HD patients (Johansen et al., 2006).

In conclusion, overweight and obesity are associated with a short-term survival advantage in HD patients. Increased LBM, better bone status, and better energy reserve stores in these patient groups may account, at least in part, for the observed protective effect of increased fat mass in epidemiological studies (Table 168.3).

Table 168.3 Potential negative and beneficial effects of increased fat mass in chronic kidney disease

Negative effects	Beneficial effects
Increased systemic inflammation	Well preserved energy stores
Dyslipidaemia	Better bone mineral density
Increased insulin resistance	Better stem cell mobilization
Obstructive sleep apnoea	More efficient disposal of lipophilic toxins
Psycho-social effects	Possible association of genetic traits (such as fetuin-A)

Obesity in peritoneal dialysis patients

Overweight and obesity have been reported to be associated with several complications of peritoneal dialysis (PD) (such as increased incidence of PD peritonitis, PD catheter loss and technique failure), and a more rapid decline of residual renal function (Piraino et al., 1991; Grzegorzewska and Mariak, 2003; McDonald et al., 2004). However, the impact of overweight on the prognosis in PD patients is less clear; there is no clear evidence so far that the 'reverse epidemiology' paradox is providing a survival advantage for PD patients with overweight. Weight gain and accumulation of fat mass is a common metabolic complication of PD and sometimes a significant clinical problem, and the aetiology is multifactorial (Box 168.2). In some PD patients, the excessive weight gain consists mainly of fat mass, located intra-abdominally (Jager et al., 2001). The glucose load from PD solutions is high but the amount of absorbed glucose (100–200 g/day) (Heimbürger et al., 1992) has not been shown to explain gain in fat mass (Konings et al., 2003). However, the glucose load in combination with obesity-predisposing gene polymorphisms, such as uncoupling protein, may result in PD-related weight gain (Nordfors et al., 2000). A relative increase of fat mass may occur over time despite of stable weight in the long term (Viglino et al., 1992). Similar to HD, PD patients who do not accumulate fat are likely to have a loss of LBM (Nordfors et al., 2000). Indeed, PD as a dialysis modality may actually be a beneficial choice for ESRD patients with low energy stores (Jager et al., 2001).

Taken together, whether overweight and obesity have any impact on mortality in PD patients is presently unclear. The mortality risk of obese PD patients is reported to be equal or increased in comparison with PD patients with normal body weight (McDonald et al., 2003), whereas some studies have reported a survival benefit for overweight and obese PD patients (Snyder et al., 2003).

Table 168.4 Components, causes and risk factors, and consequences of metabolic syndrome in chronic kidney disease

Components of metabolic syndrome	Impaired fasting blood glucose
	Insulin resistance
	Hypertension
	Dyslipidaemia (low high-density lipoprotein-cholesterol and high triglycerides)
	Abdominal obesity (in some patients)
Causes and risk factors for metabolic syndrome	Old age
	Genetic factors
	Increased total fat mass and abdominal fat
	Decline in muscle mass and muscle oxidative capacity
	Overweight and obesity
	Physical inactivity
	Uraemic toxins
	Chronic inflammation
	Oxidative stress
	Anaemia
	Metabolic acidosis
	Secondary hyperparathyroidism
Consequences of metabolic syndrome	Diabetes mellitus
	Hypertension
	Dyslipidaemia
	Cardiovascular disease
	Albuminuria
	Progress of CKD
	Muscle wasting
	Hyperuricaemia, kidney stones

Table 168.5 Prognostic implications of overweight and obesity for development of CKD and complications, and current recommendations as regards weight control

Prognostic value of overweight and obesity	Recommendations
Healthy populations: overweight and obesity are established risk factors for developing CKD	Avoiding overweight is likely beneficial in order to preserve renal function. High physical activity is recommended
CKD 1–2: cardiovascular and metabolic risk factor profile comparable to that seen in the general population	Prognostic value of weight loss in CKD 1 and 2 is not established. High physical activity is recommended. Energy intake should be scaled to normal BMI and not to actual body weight
CKD 3–5: obesity is a prognostic factor for progression of CKD throughout stages 3 and 4. LBM may be a more important determinant for survival in CKD stages 3–5 than BMI	Preservation of LBM is likely to be beneficial. High and individually modified physical activity is recommended. Energy intake should be scaled to normal BMI and not to actual body weight. Pharmacological treatment of obesity is not established
Haemodialysis: overweight and obesity are positive prognostic factors for short-term mortality. The prognostic impact on long-term mortality is not established	Preservation of LBM is beneficial. Individually modified physical activity is recommended. Energy intake should follow the current recommendations for haemodialysis patients but be scaled to normal BMI
Peritoneal dialysis: overweight and obesity is a complication of PD for some patients. The prognostic implications of overweight and obesity are unclear	Preservation of LBM is beneficial. Individually modified physical activity is recommended. Energy intake should follow the current recommendations for haemodialysis patients but be scaled to normal BMI
Kidney transplants: pre-transplantation obesity worsens the prognosis for surgical complications and graft function in the short-term whereas the long-term prognostic implications are unclear. Post-transplant weight gain increases metabolic and cardiovascular morbidity risk. Overweight and obesity are complications of immunosuppressive medication in some patients	Transplanted patients may benefit from maintaining stable and normal body weight. High physical activity is recommended. Pharmacological treatment of obesity is not established and potential interactions with the immunosuppressive medication may occur. Surgical treatment of obesity can be considered

Obesity in renal transplanted patients

Weight gain has a negative prognostic effect on the clinical outcome in renal transplant patients. Pre-transplant obesity is a risk factor for all types of surgical complications, delayed graft function, and prolonged hospitalizations in the short term after renal transplantation (Johnson et al., 1999; Espejo et al., 2003; Singh et al., 2005). Thus, many transplantation centres regard high BMI (30–35 kg/m^2) as an exclusion criterion for renal transplant recipients. The effects of pre-transplantation overweight and obesity on graft and patient long-term survival are more conflicting. Some studies report overweight and obesity as predictors for delayed graft function, and poorer graft and patient survival (Meier-Kriesche et al., 1999, 2002; Aalten et al., 2006; Sancho et al., 2007), independent of the type of the transplantation (living vs cadaveric donor) (Mehta et al., 2007). Weight gain after kidney transplantation is common and accentuated by overweight prior to transplantation (Drafts et al., 1997). Several immunosuppressive drugs have adverse effects on metabolism and predispose these patients to weight gain, diabetes, dyslipidaemia, and MetS, which may have negative effects on graft survival (Porrini et al., 2010)(Box 168.3). Whereas improved nutritional status has been reported to contribute to post-transplant weight gain (Cofan et al., 2005; Cashion et al., 2007), obesity is related to worse transplantation outcomes in other studies (Parikh et al., 2003; Armstrong et al., 2005; Ducloux et al., 2005; Chang et al., 2007). The quality of kidney transplants from obese donors is poorer than those from normal weight donors and related to higher incidence of acute allograft rejections (Massarweh et al., 2005; Espinoza et al., 2006). Whether obesity of the donor has any long-term adverse prognostic effect on graft or patient survival of the recipient is not yet established (Rea et al., 2006).

Table 168.6 Explanatory factors for the 'reverse epidemiology' paradox of obesity and for the discrepancies in studies on weight and mortality in US and European haemodialysis patients

Factors	Comment
Prevalence of obesity	The prevalence of obesity is higher in United States than Europe
Variability in ethnic groups	Obesity is more prevalent in ethnic groups, in United States (Hispanics and African Americans)
Uraemic toxin load	A larger dialysis dose in obese patients, higher V in the Kt/V index
Lean body mass	Higher in obese patients
General survival in haemodialysis	Long-term effects of obesity may not be evident due to increased general mortality in patients on haemodialysis
Length of follow-up	Short follow-up in studies. Potential negative long-term impact due to obesity may not show up

Obese donors are reported to have more perioperative surgical complications, but the long-term impact of obesity on the renal function of the renal transplant donors is not known (Pesavento et al., 1999).

Aetiology and pathogenesis of obesity-induced kidney disease

Obesity per se is a risk factor for declining renal function (Hsu et al., 2006) (Fig. 168.2). The defined histopathological changes seen are hyperfiltration, glomerular hypertrophy, minimal podocyte effacement, and segmental or global glomerulosclerosis, resembling secondary focal segmental glomerulosclerosis (FSGS) (Praga et al., 2000). The degree of glomerulomegaly correlates with the degree of obesity (Verani, 1992). To what extent weight loss reverses these changes is unclear (Morales et al., 2003). The risk of developing an obesity-related FSGS is considerable and the glomerular lesions are distinguishable from other types of FSGS (Praga et al., 2001). The histopathological changes in kidney at different stages of MetS have not been described. Declining kidney function and microalbuminuria are associated with MetS (Lea et al., 2008) and aggravate the cardiovascular risk associated with MetS. Although microalbuminuria is an established surrogate marker for endothelial dysfunction the exact mechanisms by which microalbuminuria leads to increased cardiovascular morbidity are not fully understood (Jassal et al., 2008; Vlek et al., 2008). Overweight may also aggravate the effects of an underlying kidney disease on renal function through hypertension, increased proteinuria, and obesity glomerulopathy (Bonnet et al., 2001).

Box 168.2 Risk factors for weight gain and accumulation of fat mass during peritoneal dialysis

- Overweight prior to peritoneal dialysis
- Diabetes mellitus
- Female
- Heredity for overweight
- Comorbidity
- High-transporter.

Box 168.3 Immunosuppressive drugs with adverse effects on glucose and lipid metabolism

- Corticosteroids
- Tacrolimus
- Ciclosporin
- Mycophenolate mofetil
- Sirolimus

The different components of MetS correlate more or less with each other (Hanson et al., 2002) and are prognostically related to increased cardiovascular mortality in the general population (Isomaa et al., 2001). However, the association between increased mortality and overweight may be weaker in CKD patients (Kwan et al., 2007), and confounded by other interacting risk factors (such as inflammation and proteinuria). Prevention of obesity and overweight decreases the incidence of MetS and type-2 diabetes and related complications in healthy subjects (Hanson et al., 1995). Metabolic syndrome is a risk factor for CKD in non-diabetic adults (Kurella et al., 2005; Zhang et al., 2007) and the strength of association is linearly increasing by the number of metabolic traits (Kurella et al., 2005; Zhang et al., 2007). Hypertension and obesity are a part of MetS and increase the risk for proteinuria (Lea et al., 2008). Although there is little data on the impact of MetS on CVD-related mortality at different stages of CKD, it has been shown that fat mass (estimated by BMI) is a proxy for insulin resistance in CKD stages 3–4 (Trirogoff et al., 2007).

Adipose tissue is a complex organ with functions beyond energy storage. Indeed, fat secretes a number of adipokines and cytokines. Omental (visceral) adipose tissue releases two to three times more interleukin 6 than subcutaneous fat tissue (Fried et al., 1998). Inflammation resulting from increased expression of pro-inflammatory genes has been documented in uraemic subcutaneous tissue (Witasp et al., 2011). Accumulation of adipokines (leptin, adiponectin, and visfatin) from adipose tissue has been shown to occur when renal function declines. These proteins regulate body weight and energy homeostasis, insulin resistance, dyslipidaemia, inflammation, fibrinolysis, endothelial function, and coagulation (Gaal et al., 2006) and have been suggested to play a major role in uraemic MetS even though the exact mechanisms are not known. Leptin was initially described as a central modulator of feeding behaviour (Zhang et al., 1994). Subsequent studies have demonstrated that leptin also has important peripheral effects, such as inhibition of insulin secretion, stimulation of the sympathetic nervous system, stimulation of haematopoiesis, and promotion of oxidative stress and vascular calcification. It has been suggested that the kidney is a target organ for leptin and that this hormone may play a role in renal pathophysiology, especially fibrosis (Wolf and Ziyadeh, 2006). Loss of renal function leads to inappropriately elevated serum concentrations of leptin especially in women with high BMI and those treated by PD (Heimburger et al., 1997). An association between inflammatory biomarkers and leptin in CKD has been shown and in this way leptin may

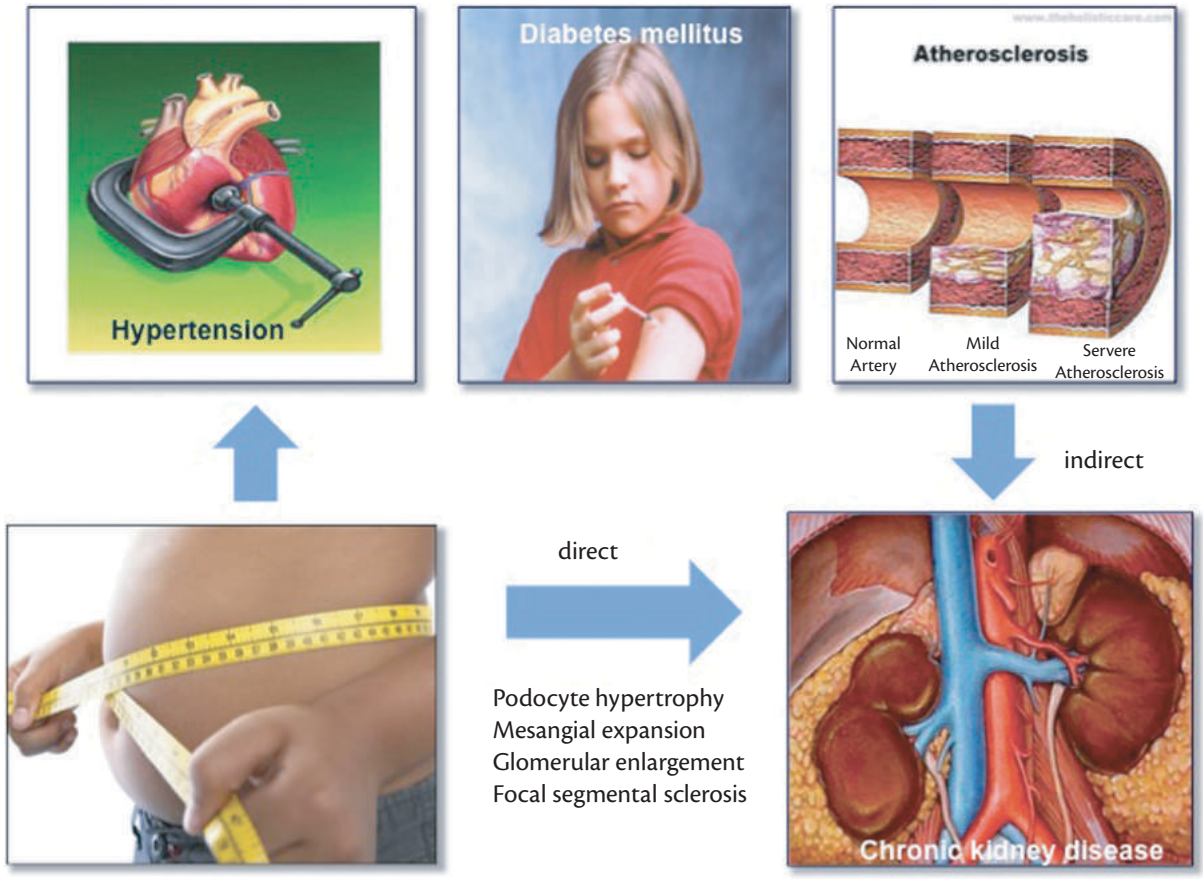

Fig. 168.2 Potential direct and indirect mechanism by which obesity may increase the risk of chronic kidney disease.

play a role in uraemic PEW (Nordfors et al., 1998). Low leptin levels are a predictor for poor outcome in dialysis patients, probably reflecting the negative effects of energy wasting and loss of fat mass (Scholze et al., 2007). Adiponectin is an adipokine inversely related to fat mass that improves insulin sensitivity, ameliorates endothelial dysfunction, and counteracts pro-inflammatory signalling (Takemura et al., 2007). However, in CKD patients, high, rather than low circulating levels of adiponectin predict mortality (Kistorp et al., 2005; Menon et al., 2006). Although the underlying mechanisms are not understood, it can be speculated that high adiponectin levels reflect a wasting process (Qi et al., 2004); in addition, direct central nervous effects promoting increased resting energy expenditure and decreased appetite may also play a role.

Treatment and outcome

Risk for obesity-related comorbidities increases linearly with increasing BMI in healthy populations (Calle et al., 1999; McGee, 2005). However, the relationship between mortality and BMI is more complex and potentially confounded by several factors (Calle et al., 1999; McGee, 2005). The association between body weight and mortality is even more complex in CKD patients than in the general population due to the abundance of shared risk factors for cardiovascular and metabolic morbidity and mortality in CKD patients. To what extent intentional weight loss influences mortality among obese healthy subjects is controversial (Williamson et al., 1995; Sorensen et al., 2005). Unintentional weight loss in CKD patients heralds poor prognosis (Kotanko et al., 2007). There is no data on whether intentional weight loss combined with increase in LBM would be beneficial in all or some subgroups of CKD patients. An open-label prospective nonrandomized intervention study evaluating a 24-month weight-management programme that included a low-fat renal-specific diet, exercise, and orlistat, a drug that prevents dietary fat from being absorbed by inhibiting gastric and pancreatic lipases, demonstrated significant weight loss and weight-loss maintenance in obese CKD patients (MacLaughlin et al., 2010). In the general population, bariatric surgery is the most effective treatment for obesity and has been shown to drastically improve both blood pressure and diabetic control without negative effects on renal function (Schuster et al., 2011). Although the interaction of bariatric surgery and renal

Box 168.4 Causes of reduced physical activity in CKD patients

- Tiredness
- Nausea
- Low muscle mass and fragility
- Comorbidities
- Medications
- Time spent on dialysis treatment.

function is less clear at different stages of CKD, a review demonstrated a significant potential for bariatric surgery to improve outcomes in CKD and in transplanted patients (Modanlou et al., 2009; Szomstein et al., 2010).

Physical inactivity relates to obesity and albuminuria (White et al., 2011). Many factors may lead to a decrease in physical activity in CKD patients (Box 168.4). Physical activity is an important tool in order to preserve or increase LBM and should be strongly encouraged for CKD patients. The effect of physical activity for LBM has not been well documented in CKD patients (Biolo et al., 2005). Current knowledge and recommendations overweight and obesity in CKD patients are presented in Table 168.5.

References

4S Study Group (1994). Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet*, 344, 1383–9.

Aalten, J., Christiaans, M. H., de Fijter, H., *et al.* (2006). The influence of obesity on short- and long-term graft and patient survival after renal transplantation. *Transpl Int*, 19(11), 901–7.

Armstrong, K. A., Campbell, S. B., Hawley, C. M., *et al.* (2005). Obesity is associated with worsening cardiovascular risk factor profiles and proteinuria progression in renal transplant recipients. *Am J Transplant*, 5(11), 2710–18.

Arner, P. (2003). The adipocyte in insulin resistance: key molecules and the impact of the thiazolidinediones. *Trends Endocrinol Metab*, 14, 137–45.

Barreto Silva, M. I., Avesani, C. M., Vale, B., *et al.* (2008). Agreement between anthropometry and bioelectrical impedance for measuring body fat in nonobese and obese nondialyzed chronic kidney disease patients. *J Ren Nutr*, 18(4), 355–62.

Biolo, G., Ciocchi, B., Stulle, M., *et al.* (2005). Metabolic consequences of physical inactivity. *J Ren Nutr*, 15(1), 49–53.

Björntorp, P. (1992). Abdominal fat distribution and disease: an overview of epidemiological data. *Ann Med*, 24(1), 15–18.

Bonnet, F., Deprele, C., Sassolas, A., *et al.* (2001). Excessive body weight as a new independent risk factor for clinical and pathological progression in primary IgA nephritis. *Am J Kidney Dis*, 37(4), 720–7.

Calle, E. E., Thun, M. J., Petrelli, J. M., *et al.* (1999). Body-mass index and mortality in a prospective cohort of US adults. *New Engl J Med*, 341, 1097–105.

Cashion, A. K., Sanchez, Z. V., Cowan, P. A., *et al.* (2007). Changes in weight during the first year after kidney transplantation. *Prog Transplant*, 17(1), 40–7.

Chang, S. H., Coates, P. T., and McDonald, S. P. (2007). Effects of body mass index at transplant on outcomes of kidney transplantation. *Transplantation*, 84(8), 981–7.

Chen, J., Muntner, P., Hamm, L. L., *et al.* (2004). The metabolic syndrome and chronic kidney disease in U.S. adults. *Ann Intern Med*, 140(3), 167–74.

Cofan, F., Vela, E., Clèries, M., *et al.* (2005). Obesity in renal transplantation: analysis of 2691 patients. *Transplant Proc*, 37(9), 3695–7.

Cordeiro, A. C., Qureshi, A. R., Stenvinkel, P., *et al.* (2010). Abdominal fat deposition is associated with increased inflammation, protein-energy wasting and worse outcome in patients undergoing haemodialysis. *Nephrol Dial Transplant*, 25(2), 562–8.

Coresh, J., Selvin, E., Stevens, L. A., *et al.* (2007). Prevalence of chronic kidney disease in the United States. *JAMA*, 298(17), 2038–47.

De Koning, L., Merchant, A. T., Pogue, J., *et al.* (2007). Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. *Evid Based Med*, 12(6), 184.

De Mutsert, R., Snijder, M. B., van der Sman-de Beer, F., *et al.* (2007). Association between body mass index and mortality is similar in the hemodialysis population and the general population at high age and equal duration of follow-up. *J Am Soc Nephrol*, 18(3), 967–74.

Drafts, H. H., Anjum, M. R., Wynn, J. J., *et al.* (1997). The impact of pre-transplant obesity on renal transplant outcomes. *Clin Transplant*, 11(5 Pt 2), 493–6.

Ducloux, D., Kazory, A., Simula-Faivre, D., *et al.* (2005). One-year post-transplant weight gain is a risk factor for graft loss. *Am J Transplant*, 5(12), 2922–8.

Ejerblad, E., Fored, C. M., Lindblad, P., *et al.* (2006). Obesity and risk for chronic renal failure. *J Am Soc Nephrol*, 17, 1695–702.

Elsayed, E. F., Tighiouart, H., Weiner, D. E., *et al.* (2008). Waist-to-hip ratio and body mass index as risk factors for cardiovascular events in CKD. *Am J Kidney Dis*, 52(1), 49–57.

Espejo, B., Torres, A., Valentín, M., *et al.* (2003). Obesity favors surgical and infectious complications after renal transplantation. *Transplant Proc*, 35(5), 1762–3.

Espinoza, R., Gracida, C., Cancino, J., *et al.* (2006). Effect of obese living donors on the outcome and metabolic features in recipients of kidney transplantation. *Transplant Proc*, 38(3), 888–9.

Fleischmann, E., Teal, N., Dudley, J., *et al.* (1999). Influence of excess weight on mortality and hospital stay in 1346 hemodialysis patients. *Kidney Int*, 55, 1560–7.

Fouque, D., Kalantar-Zadeh, K., Kopple, J., *et al.* (2008). A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. *Kidney Int*, 73(4), 391–8.

Fox, C. S., Larson, M. G., Leip, E. P., *et al.* (2004). Predictors of new-onset kidney disease in a community-based population. *JAMA*, 291(7), 844–50.

Fried, S. K., Bunkin, D. A., and Greenberg, A. S. (1998). Omental and subcutaneous adipose tissue of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrin Metab*, 83, 847–50.

Gaal, V., Mertens, I. L., and De Block, C. E. (2006). Mechanism linking obesity with cardiovascular disease. *Nature*, 444, 875–80.

Gelber, R. P., Gaziano, J. M., Manson, J. E., *et al.* (2007). A prospective study of body mass index and the risk of developing hypertension in men. *Am J Hypertens*, 20(4), 370–7.

Gelber, R. P., Kurth, T., Kausz, A. T., *et al.* (2005). Association between body mass index and CKD in apparently healthy men. *Am J Kidney Dis*, 46(5), 871–80.

Glanton, C. W., Hypolite, I. O., Hshieh, P. B., *et al.* (2003). Factors associated with improved short term survival in obese end stage renal disease patients. *Ann Epidemiol*, 13, 136–43.

Grzegorzewska, A. E. and Mariak, I. (2003). Differences in clinical and laboratory data of peritoneal dialysis patients selected according to body mass index. *Adv Perit Dial*, 19, 222–6.

Hanson, R. L., Imperatore, G., Bennett, P. H., *et al.* (2002). Components of the 'metabolic syndrome' and incidence of type 2 diabetes. *Diabetes*, 51(10), 3120–7.

Hanson, R. L., Narayan, K. M., McCance, D. R., *et al.* (1995). Rate of weight gain, weight fluctuation, and incidence of NIDDM. *Diabetes*, 44(3), 261–6.

Heimburger, O., Lonnqvist, F., Danielsson, A., *et al.* (1997). Serum immunoreactive leptin concentration and its relation to the body fat content in chronic renal failure. *J Am Soc Nephrol*, 8(9), 1423–30.

Heimburger, O., Qureshi, A. R., Blaner, W. S., *et al.* (2000). Hand-grip muscle strength, lean body mass, and plasma proteins as markers of nutritional status in patients with chronic renal failure close to start of dialysis therapy. *Am J Kidney Dis*, 36(6), 1213–25.

Heimbürger, O., Waniewski, J., Werynski, A., *et al.* (1992). A quantitative description of solute and fluid transport during peritoneal dialysis. *Kidney Int*, 41, 1320–32.

Honda, H., Qureshi, A. R., Axelsson, J., *et al.* (2007). Obese sarcopenia in end-stage renal disease patients is associated with inflammation and increased mortality. *Am J Clin Nutr*, 86(3), 633–8.

Hsu, C. Y., McCulloch, C. E., Iribarren, C., *et al.* (2006). Body mass index and risk for end-stage renal disease. *Ann Intern Med*, 144, 21–8.

Huang, C. X., Tighiouart, H., Beddhu, S., *et al.* (2010). Both low muscle mass and low fat are associated with higher all-cause mortality in hemodialysis patients. *Kidney Int*, 77(7), 624–9.

Iseki, K. (2006). Body mass index and the risk of chronic renal failure: the Asian experience. *Contrib Nephrol*, 151, 42–56.

Isomaa, B., Almgren, P., Tuomi, T., *et al.* (2001). Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care*, 24(4), 683–9.

Jager, K. J., Merkus, M. P., Huisman, R. M., *et al.* (2001). Nutritional status over time in haemodialysis and peritoneal dialysis. *J Am Soc Nephrol*, 12, 1272–9.

Jassal, S. K., Langenberg, C., von Mühlen, D., *et al.* (2008). Usefulness of microalbuminuria versus the metabolic syndrome as a predictor of cardiovascular disease in women and men>40 years of age (from the Rancho Bernardo Study). *Am J Cardiol*, 101(9), 1275–80.

Johansen, K. L., Kutner, N. G., Young, B., *et al.* (2006). Association of body size with health status in patients beginning dialysis. *Am J Clin Nutr*, 83, 543–9.

Johnson, C. P., Kuhn, E. M., Hariharan, S., *et al.* (1999). Pre-transplant identification of risk factors that adversely affect length of stay and charges for renal transplantation. *Clin Transplant*, 13(2), 168–75.

Kalantar-Zadeh, K., Abbott, K. C., Salahudeen, A. K., *et al.* (2005). Survival advantages of obesity in dialysis patients. *Am J Clin Nutr*, 81(3), 543–54.

Kalantar-Zadeh, K., Block, G., Humphreys, M. H., *et al.* (2003). Reverse epidemiology of cardiovascular risk factors in maintenance dialysis patients. *Kidney Int*, 63, 793–808.

Kamimura, M. A., Avesani, C. M., Cendoroglo, M., *et al.* (2003). Comparison of skinfold thicknesses and bioelectrical impedance analysis with dual-energy X-ray absorptiometry for the assessment of body fat in patients on long-term haemodialysis therapy. *Nephrol Dial Transplant*, 18(1), 101–5.

Keith, D. S., Nichols, G. A., Gullion, C. M., *et al.* (2004). Longitudinal follow-up and outcomes among a population with chronic kidney disease in a large managed care organization. *Arch Intern Med*, 164, 659–63.

Kistorp, C., Faber, J., Galatius, S., *et al.* (2005). Plasma adiponectin, body mass index and mortality in patients with chronic heart failure. *Circulation*, 112, 1756–62.

Konings, C. J., Kooman, J. P., Schonck, M., *et al.* (2003). Fluid status in CAPD patients is related to peritoneal transport and residual renal function: evidence from a longitudinal study. *Nephrol Dial Transpl*, 18, 797–803.

Kotanko, P., Thijssen, S., Kitzler, T., *et al.* (2007). Size matters: body composition and outcomes in maintenance hemodialysis patients. *Blood Purif*, 25, 27–30.

Kramer, H., Toto, R., Peshock, R., *et al.* (2005). Association between chronic kidney disease and coronary artery calcification: the Dallas Heart Study. *J Am Soc Nephrol*, 16, 507–13.

Kurella, M., Lo, J. C., and Chertow, G. M. (2005). Metabolic syndrome and the risk for chronic kidney disease among nondiabetic adults. *J Am Soc Nephrol*, 16, 2134–40.

Kwan, B. C., Murtaugh, M. A., and Beddhu, S. (2007). Associations of body size with metabolic syndrome and mortality in moderate chronic kidney disease. *Clin J Am Soc Nephrol*, 2(5), 992–8.

Lea, J., Cheek, D., Thornley-Brown, D., *et al.* (2008). Metabolic syndrome, proteinuria, and the risk of progressive CKD in hypertensive African Americans. *Am J Kidney Dis*, 51(5), 732–40.

Lee, C. C., Sun, C. Y., Wu, I. W., *et al.* (2011). Metabolic syndrome loses its predictive power in late-stage chronic kidney disease progression—a paradoxical phenomenon. *Clin Nephrol*, 75(2), 141–9.

Luk, A. O., So, W. Y., Ma, R. C., *et al.* (2008). Metabolic syndrome predicts new onset of chronic kidney disease in 5,829 patients with type 2 diabetes: a 5-year prospective analysis of the Hong Kong Diabetes Registry. *Diabetes Care*, 31(12), 2357–61.

MacLaughlin, H. L., Cook, S. A., Kariyawasam, D., *et al.* (2010). Nonrandomized trial of weight loss with orlistat, nutrition education, diet, and exercise in obese patients with CKD: 2-year follow-up. *Am J Kidney Dis*, 55(1), 69–76.

Madero, M., Sarnak, M. J., Wang, X., *et al.* (2007). Body mass index and mortality in CKD. *Am J Kidney Dis*, 50, 404–11.

Massarweh, N. N., Clayton, J. L., Mangum, C. A., *et al.* (2005). High body mass index and short- and long-term renal allograft survival in adults. *Transplantation*, 80(10), 1430–4.

McDonald, S. P., Collins, J. F., and Johnson, D. W. (2003). Obesity is associated with worse peritoneal dialysis outcomes in the Australia and New Zealand patient populations. *J Am Soc Nephrol*, 14(11), 2894–901.

McDonald, S. P., Collins, J. F., Rumpsfeld, M., *et al.* (2004). Obesity is a risk factor for peritonitis in the Australian and New Zealand peritoneal dialysis patient populations. *Perit Dial Int*, 24(4), 340–6.

McGee, D. L. (2005). Body mass index and mortality: a meta-analysis based on person-level data from twenty-six observational studies. *Ann Epidemiol*, 15(2), 87–97.

Mehta, R., Shah, G., Leggat, J. E., *et al.* (2007). Impact of recipient obesity on living donor kidney transplant outcomes: a single-center experience. *Transplant Proc*, 39(5), 1421–3.

Meier-Kriesche, H. U., Arndorfer, J. A., and Kaplan, B. (2002). The impact of body mass index on renal transplant outcomes: a significant independent risk factor for graft failure and patient death. *Transplantation*, 73(1), 70–4.

Meier-Kriesche, H. U., Vaghela, M., Thambuganipalle, R., *et al.* (1999). The effect of body mass index on long-term renal allograft survival. *Transplantation*, 68(9), 1294–7.

Menon, V., Li, L., Wang, X., *et al.* (2006). Adiponectin and mortality in patients with chronic kidney disease. *J Am Soc Nephrol*, 17, 2599–606.

Modanlou, K. A., Muthyala, U., Xiao, H., *et al.* (2009). Bariatric surgery among kidney transplant candidates and recipients: analysis of the United States renal data system and literature review. *Transplantation*, 87(8), 1167–73.

Morales, E, Valero, M. A., León, M., *et al.* (2003). Beneficial effects of weight loss in overweight patients with chronic proteinuric nephropathies. *Am J Kidney Dis*, 41, 319–27.

Muntner, P., Winston, J., Uribarri, J., *et al.* (2008). Overweight, obesity, and elevated serum cystatin C levels in adults in the United States. *Am J Med*, 121(4), 341–8.

Nordfors, L., Heimburger, O., Lönnqvist, F., *et al.* (2000). Fat tissue accumulation during peritoneal dialysis is associated with a polymorphism in uncoupling protein 2. *Kidney Int*, 57(4), 1713–9.

Nordfors, L., Lonnqvist, F., Heimbürger, O., *et al.* (1998). Low leptin gene expression and hyperleptinemia in chronic renal failure. *Kidney Int*, 54(4), 1267–75.

Parikh, C. R., Klem, P., Wong, C., *et al.* (2003). Obesity as an independent predictor of posttransplant diabetes mellitus. *Transplant Proc*, 35(8), 2922–6.

Park, J. T., Chang, T. I., Kim, D. K., *et al.* (2010). Metabolic syndrome predicts mortality in non-diabetic patients on continuous ambulatory peritoneal dialysis. *Nephrol Dial Transplant*, 25(2), 599–604.

Pesavento, T. E., Henry, M. L., Falkenhain, M. E., *et al.* (1999). Obese living kidney donors: short-term results and possible implications. *Transplantation*, 68(10), 1491–6.

Piraino, B., Bernardini, J., Centa, P. K., *et al.* (1991). The effect of body weight on CAPD related infections and catheter loss. *Perit Dial Int*, 11(1), 64–8.

Porrini, E., Delgado, P., and Torres, A. (2010). Metabolic syndrome, insulin resistance, and chronic allograft dysfunction. *Kidney Int Suppl*, 119, S42–6.

Postorino, M., Marino, C., Tripepi, G., *et al.* (2011). Abdominal obesity modifies the risk of hypertriglyceridemia for all-cause and cardiovascular mortality in hemodialysis patients. *Kidney Int*, 79(7), 765–72.

Praga, M., Hernández, E., Herrero, J. C., *et al.* (2000). Influence of obesity on the appearance of proteinuria and renal insufficiency after unilateral nephrectomy. *Kidney Int*, 58, 2111–18.

Praga, M., Hernández, E., Morales, E., *et al.* (2001). Clinical features and long-term outcome of obesity-associated focal segmental glomerulosclerosis. *Nephrol Dial Transplant*, 16, 1790–8.

Qi, Y., Takahashi, N., Hileman, S. M., *et al.* (2004). Adiponectin acts in the brain to decrease body weight. *Nature Med*, 10, 524–9.

Ramkumar, N., Pappas, L. M., and Beddhu, S. (2005). Effect of body size and body composition on survival in peritoneal dialysis patients. *Perit Dial Int*, 25(5), 461–9.

Rea, D. J., Heimbach, J. K., Grande, J. P., *et al.* (2006). Glomerular volume and renal histology in obese and non-obese living kidney donors. *Kidney Int*, 70(9), 1636–41.

Sancho, A., Avila, A., Gavela, E., *et al.* (2007). Effect of overweight on kidney transplantation outcome. *Transplant Proc*, 39(7), 2202–4.

Scholze, A., Rattensperger, D., Zidek, W., *et al.* (2007). Low serum leptin predicts mortality in patients with chronic kidney disease stage 5. *Obesity*, 15, 1617–22.

Schuster, D. P., Teodorescu, M., Mikami, D., *et al.* (2011). Effect of bariatric surgery on normal and abnormal renal function. *Surg Obes Relat Dis*, 7(4), 459–64.

Singh, D., Lawen, J., and Alkhudair, W. (2005). Does pretransplant obesity affect the outcome in kidney transplant recipients? *Transplant Proc*, 37(2), 717–20.

Snyder, J. J., Foley, R. N., Gilbertson, D. T., *et al.* (2003). Body size and outcomes on peritoneal dialysis in the United States. *Kidney Int*, 64(5), 1838–44.

Sorensen, T. I., Rissanen, A., Korkeila, M., *et al.* (2005). Intention to lose weight, weight changes, and 18-year mortality in overweight individuals without co-morbidities. *PLoS Med*, 2(6), e171.

Stack, A. G., Murthy, B. V., and Molony, D. A. (2004). Survival differences between peritoneal dialysis and hemodialysis among large ESRD patients in the United States. *Kidney Int*, 65(6), 2398–408.

Szomstein, S., Rojas, R., and Rosenthal, R. J. (2010). Outcomes of laparoscopic bariatric surgery after renal transplant. *Obes Surg*, 20(3), 383–5.

Takemura, Y., Walsh, K., and Ouchi, N. (2007). Adiponectin and cardiovascular inflammatory responses. *Curr Atheroscler Rep*, 9(3), 238–43.

Thomas, G., Sehgal, A. R., Kashyap, S. R., *et al.* (2011). Metabolic syndrome and kidney disease: a systematic review and meta-analysis. *Clin J Am Soc Nephrol*, 6(10), 2364–73.

Trirogoff, M. L., Shintani, A., Himmelfarb, J., *et al.* (2007). Body mass index and fat mass are the primary correlates of insulin resistance in nondiabetic stage 3–4 chronic kidney disease patients. *Am J Clin Nutr*, 86(6), 1642–8.

Velludo, C. M. O., Kamimura, M. A., Avesani, C. M., *et al.* (2008). Reproductibility of waist circumference in chronic kidney disease patients. *J Renal Nutr*, 18, S43.

Verani, R. R. (1992). Obesity-associated focal segmental glomerulosclerosis: pathological features of the lesion and relationship with cardiomegaly and hyperlipidemia. *Am J Kidney Dis*, 20(6), 629–34.

Viglino, G., Gallo, M., Cottino, R., *et al.* (1992). Assessment of nutritional status of CAPD patients during a five-year follow up. In K. Ota, J. Maher, J. Winchester, *et al.* (eds.) *Current Concepts in Peritoneal Dialysis*, pp. 497–505. Amsterdam: Elsevier Science Publishers.

Vlek, A. L., van der Graaf, Y., Spiering, W., *et al.* (2008). Cardiovascular events and all-cause mortality by albuminuria and decreased glomerular filtration rate in patients with vascular disease. *J Intern Med*, 264(4), 351–60.

Wang, Y., Chen, X., Song, Y., *et al.* (2008). Association between obesity and kidney disease: A systematic review and meta-analysis. *Kidney Int*, 73, 19–33.

White, S. L., Dunstan, D. W., Polkinghorne, K. R., *et al.* (2011). Physical inactivity and chronic kidney disease in Australian adults: the AusDiab study. *Nutr Metab Cardiovasc Dis*, 21(2), 104–12.

Williamson, D. F., Pamuk, E., Thun, M., *et al.* (1995). Prospective study of intentional weight loss and mortality in never-smoking overweight US white women aged 40–64 years. *Am J Epidemiol*, 141(12), 1128–41.

Witasp, A., Carrero, J. J., Heimbürger, O., *et al.* (2011). Increased expression of pro-inflammatory genes in abdominal subcutaneous fat in advanced chronic kidney disease patients. *J Intern Med*, 269(4), 410–9.

Wolf, G. and Ziyadeh, F. N. (2006). Leptin and renal fibrosis. *Contrib Nephrol*, 151, 175–83.

World Health Organization (2011). *Obesity and overweight. Fact sheet N°311*. <http://www.who.int/mediacentre/factsheets/fs311/en/index.html>

Zhang, L., Zuo, L., Wang, F., *et al.* (2007). Metabolic syndrome and chronic kidney disease in a Chinese population aged 40 years and older. *Mayo Clin Proc*, 82(7), 822–7.

Zhang, Y., Proenca, R., Maffei, M., *et al.* (1994). Positional cloning of the mouse obese gene and its human homlogue. *Nature*, 372(6505), 425–32.

Zoccali, C., Postorino, M., Marino, C., *et al.* (2011). Waist circumference modifies the relationship between the adipose tissue cytokines leptin and adiponectin and all-cause and cardiovascular mortality in haemodialysis patients. *J Intern Med*, 269(2), 172–81.

CHAPTER 169

The patient with hepatorenal syndrome

Andrés Cárdenas and Pere Ginès

Introduction

Renal failure in patients with cirrhosis was initially described as a renal dysfunction characterized by progressive azotaemia associated with marked abnormalities of the systemic arterial circulation and normal renal histology, a condition later coined as hepatorenal syndrome (HRS) (Hecker and Sherlock, 1956; Lancestremere et al., 1962; Shear et al., 1965; Schroeder et al., 1967). However, it is now known that patients with cirrhosis may also develop renal failure due to a variety of causes including bacterial infections, gastrointestinal (GI) bleeding, use of non-steroidal anti-inflammatory drugs (NSAIDs), and intrinsic renal diseases (Ginès et al., 2007). The initial management of a patients with a rise in serum creatinine completely depends on the cause therefore the most important step in managing renal failure in a patient with cirrhosis is to identify its aetiology. The aim of this chapter is to review the current approach to renal failure in patients with cirrhosis, with particular emphasis on the pathogenesis, diagnosis, and management of HRS.

Diagnostic criteria

The diagnosis of renal failure in patients with cirrhosis is established when serum creatinine increases to > 1.5 mg/dL (133 μmol/L) which corresponds to a glomerular filtration rate (GFR) of approximately 30 mL/min (Ginès et al., 2009; European Association for the Study of the Liver, 2010). Serum creatinine is the most accepted marker of GFR estimation in cirrhosis, but it has important drawbacks (Francoz et al., 2010). Serum creatinine may overestimate GFR mainly due either to decreased creatinine production or reduced muscle mass. Additionally, a number of studies indicate that serum creatinine < 1.4 mg/dL does not necessarily exclude renal dysfunction (Papadakis and Areiff, 1987; Caregaro et al., 1994; Proulx et al., 2005; Francoz et al., 2010). Creatinine clearance also overestimates GFR and is difficult to perform because it depends on the adequate collection of urine volume over 24 hours, which in many cases is inadequate. The estimation of GFR with the Cockcroft and Gault (1976) and Modification of Diet in Renal Disease (MDRD) equations are based on serum creatinine and therefore have not shown to be very useful in cirrhosis (Gonwa et al., 2004; Francoz et al., 2010). The gold standard for measuring GFR in cirrhosis relies on clearance techniques of exogenous markers which allow the precise estimation of GFR. Inulin has been the most widely used marker as it is completely filtered by the glomerulus without being secreted, reabsorbed, synthesized, or metabolized by the ducts. Thus, after an intravenous (IV) infusion and at a stable concentration in healthy subjects, the amount filtered equals to the amount excreted in urine. However, this method is burdensome, expensive and not available in all settings. Other markers such as radiolabelled compounds (^{51}Cr-EDTA, ^{99m}Tc-DPTA, and ^{125}I-iothalamate) or iohexol/iothalamate are commonly used in some centres but they are expensive and expose the patient to radiation if used repeatedly.

New attempts at defining renal failure in cirrhosis using the acute kidney injury (AKI) definition have been proposed (Wong et al., 2011; Angeli et al., 2015). The proposed definition of AKI in cirrhosis is an increase in serum creatinine of > 50% from baseline or a rise in serum creatinine of ≥ 26.4 μmol/L (≥ 0.3 mg/dL) in < 48 hours, irrespective of whether the cause of the acute deterioration in renal function is related to a functional or structural disorder (Wong et al., 2011). A serum creatinine within the last 3 months before admission is considered a baseline value for the diagnosis of AKI when a value within the previous 7 days is not available. Although a single measurement of serum creatinine alone is probably inadequate for identifying and/or quantifying either acute or chronic renal disease in cirrhosis, repeated measurements over time may be useful in indicating variations of GFR in clinical practice.

Aetiology and epidemiology

There are a variety of causes of renal failure in cirrhosis (Table 169.1). These aetiologies are mainly classified as (1) renal failure associated with infections, (2) hypovolaemia-induced renal failure, (3) intrinsic renal diseases, (4) HRS, and (5) drug-induced renal failure. In patients with cirrhosis and ascites, the 1-year probability of developing HRS ranges between 18% and 23% at 1 year and increases up to 40–50% at 3–5 years of follow-up (Ginès et al., 1993; Montoliu et al., 2010) (Fig. 169.1). A systematic review of 74 studies showed that the overall median mortality in patients with cirrhosis and renal failure was 67%, with 58% mortality at 30 days and 63% mortality at 1 year (Fede et al., 2012a). In a large analysis of 562 hospitalized patients with cirrhosis and renal failure, the most frequent cause was that associated with bacterial infections (46%), followed by hypovolaemia-induced renal failure (32%), HRS (13%), parenchymal nephropathy (9%), drug-induced renal failure (7.5%), mixed causes (8%), and other causes (2%) (Martin-Llahi et al., 2011). Patients with intrinsic renal disease had a 73% survival at 3 months followed by a 46% survival in those with hypovolaemia-related renal failure. Those with renal failure associated with infections and HRS had the lowest 3-month survival probability, 31% and 15% respectively (Martin-Llahi et al., 2011) (Fig. 169.2).

Table 169.1 Main causes of renal failure in patients with cirrhosis

1. Infections
a. Spontaneous bacterial peritonitis
b. Spontaneous bacteraemia
c. Urinary tract infection, pneumonia, skin infections
2. Hypovolaemia-induced renal failure
a. Vomiting, diarrhoea
b. Gastrointestinal bleeding (with or without shock)
c. Diuretic-induced
3. Hepatorenal syndrome
4. Intrinsic renal diseases
a. Glomerulopathies—IgA nephropathy, membranous nephropathy, membranoproliferative glomerulonephritis, polyarteritis nodosa, cryoglobulinaemia due to viral hepatitis, or alcohol
b. Chronic kidney diseases due to diabetes, hypertension, or other causes
5. Drug-induced renal failure
a. Haemodynamically induced—non-steroidal anti-inflammatory agents, angiotensin receptor blockers
b. Acute tubular necrosis—aminoglycosides, amphotericin B, tenofovir, adefovir
c. Acute interstitial nephritis—penicillin, rifampin, and sulphonamides

Diagnostic approach

Once a diagnosis is secured, it is essential to establish the aetiology of renal failure before considering further management. Unfortunately there are no definite tests that tease out the different types of renal failure in cirrhosis. In most cases a detailed clinical history, physical exam, and assessment of renal function with a thorough evaluation of urine and serum electrolytes will, in many cases, suffice for establishing the cause. A stepped care approach to renal failure in cirrhosis should be performed following a checklist where essential data must be obtained from the history, physical exam, laboratory data, and a renal ultrasound (Table 169.2). Renal ultrasonography needs to be performed in order to rule out the abnormalities in renal structure suggestive of chronic kidney disease or urinary tract obstruction. Data on biomarkers in the assessment of renal failure in cirrhosis is limited and therefore cannot be routinely recommended in the workup of renal failure in cirrhosis. Since the diagnosis of HRS cannot be made with a specific test, its confirmation is currently made using criteria to exclude other causes of renal failure that can occur in cirrhosis. So, if after an extensive investigation there is no evidence of infection, hypovolaemia, administration of nephrotoxic drugs, or intrinsic renal diseases, then HRS should be considered the cause of renal failure. The diagnosis of HRS is based on specific clinical criteria that aim to exclude other causes of renal failure that are not functional (Table 169.3)—see below.

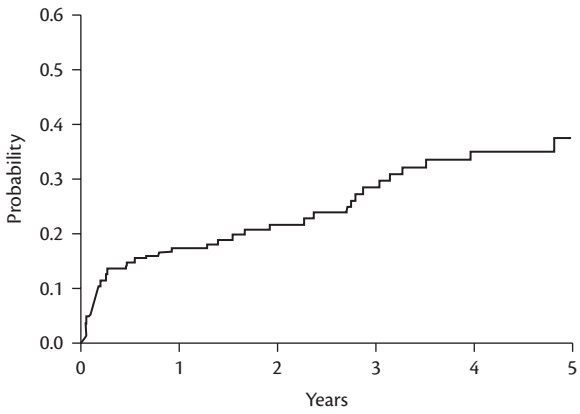

Fig. 169.1 Probability of developing hepatorenal syndrome in a series of patients with cirrhosis and ascites.
Reproduced with permission from Ginès, A., Escorsell, A., Ginès, P., *et al.* (1993). Incidence, predictive factors, and prognosis of the hepatorenal syndrome in cirrhosis with ascites. *Gastroenterology*, 105, 229–36.

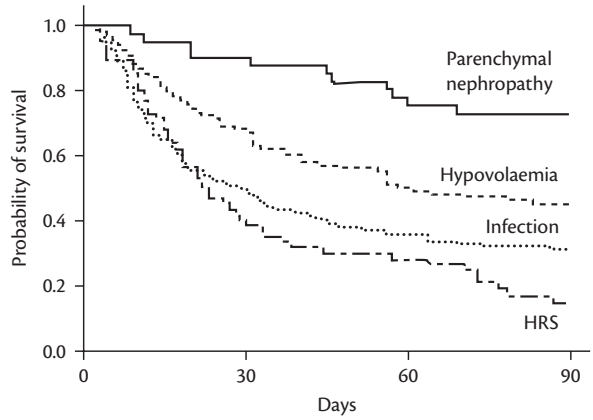

Fig. 169.2 Survival of patients with renal failure and cirrhosis according to aetiology of kidney disease. Patients with intrinsic renal disease had a 73% survival at 3 months followed by a 46% survival in those with hypovolaemia-related renal failure. Those with renal failure associated with infections and HRS had the lowest 3-month probability, 31% and 15% respectively.
Reproduced with permission from Martin-Llahi, M., Guevara, M., Torre, A., *et al.* (2011). Prognostic importance of the cause of renal failure in patients with cirrhosis. *Gastroenterology*, 140, 488–96.

Bacterial infections

These are the most common cause of renal failure in cirrhosis. The pathogenesis is related to an impairment of the systemic arterial vasodilation present in cirrhosis due to bacterial products, cytokines, or vasoactive mediators that appear in relation with the infection (Tandon and Garcia-Tsao, 2008). This occurs mainly in patients that develop spontaneous bacterial peritonitis (SBP) and spontaneous bacteraemia, but may occur with any bacterial infection (Fasolato et al, 2007; Montoliu et al., 2010; Martin-Llahi et al., 2011). It is estimated that bacterial infections are present at admission or during hospitalization in 30–60% of patients. Of these, most are secondary to SBP; other common causes are urinary tract infection, pneumonia and bacteraemia (Fernández et al., 2002; Fasolato et al., 2007) which are due to both Gram-negative bacteria and aerobic Gram-positive bacteria. Approximately one-third of infections are community acquired, one-third healthcare associated, and one-third nosocomial (Fernández et al., 2012). Unfortunately in those that are nosocomial and have positive cultures up to 35% are multiresistant to standard antibiotics (Fernández et al., 2012). Thus

Table 169.2 Initial diagnostic checklist for patients with cirrhosis presenting with renal failure

Complete history to rule out:
Infections/sepsis
NSAID use, high doses of diuretics or other drugs
GI fluid losses and GI bleed
Diabetes, arterial hypertension
Urinary obstruction
Physical examination to document:
Volume status
Haemodynamics
Signs of infection
Laboratory data:
Routine blood count, serum creatinine, electrolytes and liver tests
Regular urine analysis, urine electrolytes, and sediment
24-hour urine volume, sodium, protein
Culture—ascites, blood, urine
Chest X-ray:
Rule out pneumonia and pleural effusion
Renal ultrasound:
Rule out obstructive uropathy or chronic kidney disease

all efforts should be geared towards isolating the underlying cause of infection in these patients. Bacterial infections significantly increase mortality in patients with cirrhosis. A meta-analysis of 178 studies estimated that the overall mortality of infected patients with cirrhosis was 38% (Arvanti et al., 2010). The same analysis concluded that the mortality in those with SBP was 44% and in those with bacteraemia, the mortality was 42%. In any patient with cirrhosis that develops renal failure, the presence of a bacterial infection should be meticulously sought after. This is particularly true because the classic signs and symptoms of infection are not always present and may be even absent in some patients with cirrhosis. The mandatory workup consists of a complete blood cell count, liver chemistries, polymorphonuclear cell count in a sample of ascitic fluid, ascitic fluid cultures, urine sediment and culture, abdominal ultrasound, chest X-ray, and blood cultures.

Table 169.3 Diagnostic criteria of hepatorenal syndrome in cirrhosis

1. Cirrhosis with ascites
2. Serum creatinine > 1.5 mg/dL (133 µmol/L)
3. No improvement of serum creatinine (decrease to a level < 1.5 mg/dL (133 µmol/L) (after at least 2 days off diuretics and volume expansion with albumin (1 g/kg body weight up to a maximum of 100 g/day)
4. Absence of shock
5. No current or recent treatment with nephrotoxic drugs
6. Absence of signs of parenchymal renal disease, as suggested by proteinuria (> 500 mg/day) or haematuria (> 50 red blood cells per high power field), and/or abnormal renal ultrasound.

From Salerno Gerbes, A., Wong, F., *et al*. (2007). Diagnosis, prevention and treatment of the hepatorenal syndrome in cirrhosis. A consensus workshop of the international ascites club. *Gut*, 56, 1310–8.

Hypovolaemia

In hypovolaemia-induced renal failure the major mechanism responsible for renal hypoperfusion is a reduction in intravascular volume which, if severe, may lead to acute tubular necrosis (ATN) and a significant decrease of GFR (Ginès and Schrier, 2009). The main causes of hypovolaemia in cirrhosis are GI bleeding and over-diuresis due to excessive diuretic treatment as well as GI fluid losses due to vomiting and/or diarrhoea. In most cases, renal function improves after elimination of the precipitating cause and plasma volume expansion. Patients need to be thoroughly questioned about GI bleeding, and physical examination should include a rectal exam to rule out melena, bright red blood per rectum, or occult blood if GI bleeding is suspected. A nasogastric tube should be considered if there is a high suspicion of upper GI bleeding. Additionally, if patients were taking diuretics, marked weight loss during the days following their use suggests that they are the cause of renal failure. Patients with cirrhosis and GI bleeding require an important reduction in blood volume which in most cases is associated with hypovolaemic shock in order to develop renal failure (Cardenas et al., 2001). In moderate to severe cases of GI bleeding (those requiring > 3 units of packed red blood cells), there is a 20% chance of developing renal failure. In mild cases this figure drops to < 5% (Cardenas et al., 2001). When hypovolaemic shock develops in patients with cirrhosis and GI bleeding, there is a 60% probability of developing renal failure and mortality rates are very high.

Intrinsic renal diseases

Most intrinsic renal diseases are related to common aetiologic factors of cirrhosis, including chronic hepatitis B or C infection or alcoholic liver disease. Most encompass kidney diseases secondary to the deposition of circulating immunocomplexes in the glomeruli. The most common in hepatitis C are membranoproliferative glomerulonephritis, membranous glomerulonephritis, and focal segmental glomerular sclerosis (Lhotta, 2002). Membranous nephropathy is commonly encountered in patients with hepatitis B and immunoglobulin (Ig)-A nephropathy in patients with alcoholic cirrhosis. In some patients, the glomerular deposits are mild and do not affect kidney function, whereas in other patients they are so severe that GFR markedly decreases and renal failure develops. In patients with cirrhosis and renal failure due to intrinsic renal diseases, there is usually proteinuria or haematuria. Intrinsic renal disease is considered if there is either proteinuria > 500 mg/24 hours, abnormal urine sediment with > 50 red cells per high power field, or abnormal renal ultrasound findings in the absence of other causes of renal failure (Ginès and Schrier, 2009). However some patients with renal failure due to chronic kidney disease may not necessarily meet the definition. Therefore, criteria for this definition are specific but lack sensitivity and thus need to be properly studied.

The role of kidney biopsy in the evaluation of renal failure in cirrhosis of unclear aetiology may be useful in selected cases. Findings demonstrating glomerulosclerosis or significant fibrosis in the renal parenchyma may require that the patient receive dialysis or a simultaneous liver–kidney transplant (if being considered for liver

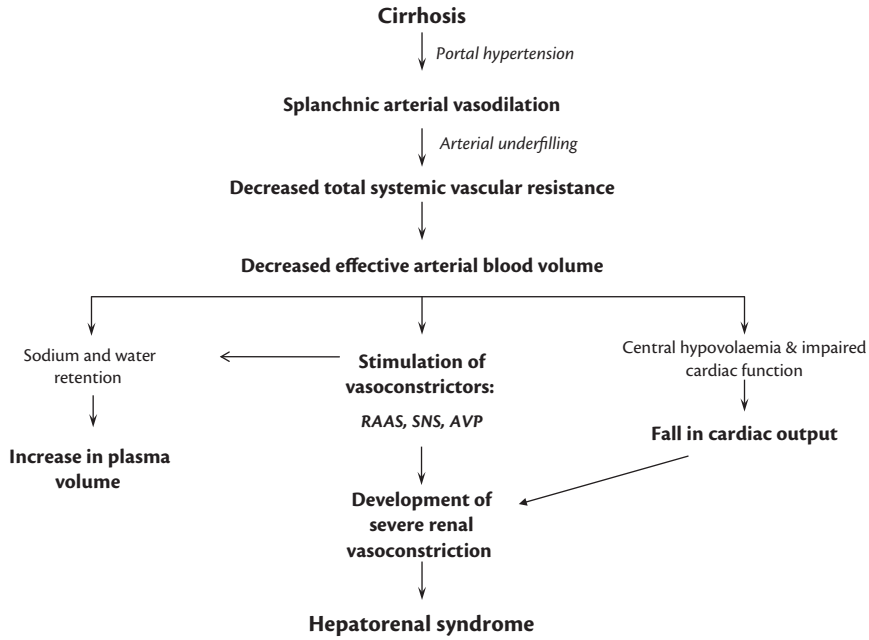

Fig. 169.3 Proposed pathogenic mechanism of hepatorenal syndrome. Splanchnic vasodilation arising from portal hypertension, an increased plasma volume and a decreased cardiac output seem to play an equally important role in the decreased renal perfusion leading to HRS. The impairment effective arterial blood volume responsible for the activation of vasoconstrictor systems acting on renal circulation is a consequence of both a low systemic vascular resistance causing an abnormal distribution of blood volume and a low cardiac output relative to the markedly dilated arterial bed secondary to cirrhotic cardiomyopathy. AVP = arginine vasopressin; RAAS = renin–angiotensin–aldosterone system; SNS = sympathetic nervous system.

transplantation (LT)). In one retrospective study of 65 patients, renal biopsy showed the presence of glomerular, vascular, and tubulointerstitial changes in 77%, 69%, and 94% of cases respectively (Trawale et al., 2010). Fibrous endarteritis was the most common renal vascular lesion and pathological changes to different structures were frequently combined. There were no reported complications of transvenous renal biopsy. Another report of 44 liver transplant candidates with renal failure of undetermined aetiology that underwent percutaneous renal biopsy revealed that IgA nephropathy and ATN were the most common findings; however, there was more than one pathological finding (i.e. interstitial fibrosis, glomerular sclerosis, or membranoproliferative disease) in 64% of patients (Wadei et al., 2008). An unfortunate finding was the development of serious complications (mainly bleeding) requiring intervention in 18% of patients. Therefore the role of renal biopsy in patients with cirrhosis still needs be studied.

Hepatorenal syndrome

HRS is a prerenal renal failure without any identifiable kidney pathology that occurs in patients with advanced cirrhosis (Arroyo et al., 1996; Salerno et al., 2007). There are two types of HRS: type 1, a rapidly progressive form of renal failure with a grim prognosis, and type 2, a steadily progressive form of renal failure with a better short-term prognosis than type 1 (see below). Patients with very advanced liver disease display a profound disturbance in the systemic circulation, characterized by a low arterial blood pressure, high cardiac output, and a decreased total systemic vascular resistance (Schrier et al., 1988). This haemodynamic pattern is due to an intense arterial vasodilation occurring in the splanchnic vascular bed that triggers a neurohormonal response with the activation of the renin–angiotensin-aldosterone system, the sympathetic nervous system, and the non-osmotic release of arginine vasopressin (the antidiuretic hormone) aimed at maintaining arterial pressure that eventually leads to vasoconstriction in several vascular beds, particularly the kidneys, decreasing the GFR (Fig. 169.3). As the disease progresses, splanchnic vasodilation worsens and this creates a vicious cycle that favours further activation of vasoconstrictors (renin, noradrenalin (norepinephrine), and vasopressin), and ongoing renal vasoconstriction that establishes HRS. Although the kidney tries to counterbalance the effects of this vasoconstriction with the intrarenal production of vasodilators such as prostaglandins, the incessant overproduction of vasoconstrictor factors overcomes their protective role and HRS ensues. In the early stages of cirrhosis, there is an increase in cardiac output that compensates for a mild decrease in systemic vascular resistance, but in very advanced cirrhosis there is marked reduction in systemic vascular resistance for which the increased cardiac output cannot compensate for with the development arterial underfilling of circulation (Arroyo and Fernández, 2011). Additionally, in the very advanced stages of cirrhosis, cardiac output drops which contributes to decreased effective arterial blood volume and decreased renal perfusion (Ruiz-del-Arbol et al., 2003, 2005; Krag et al., 2010). In recent years, several studies indicate that adrenal insufficiency (AI) in compensated and decompensated cirrhosis occurs in 26–64% of patients (Fede et al., 2012b). In those with ascites, it might play a role in the pathogenesis of HRS. Normal adrenal function is essential for an adequate response of the arterial circulation to endogenous vasoconstrictors, thus AI could be an important contributory mechanism of circulatory dysfunction associated with renal failure induced by bacterial infections. In one study, AI was found in 80% of patients with HRS but only in 34% with serum creatinine < 1.5 mg/dL (Tsai et al., 2006). Another study showed that in patients

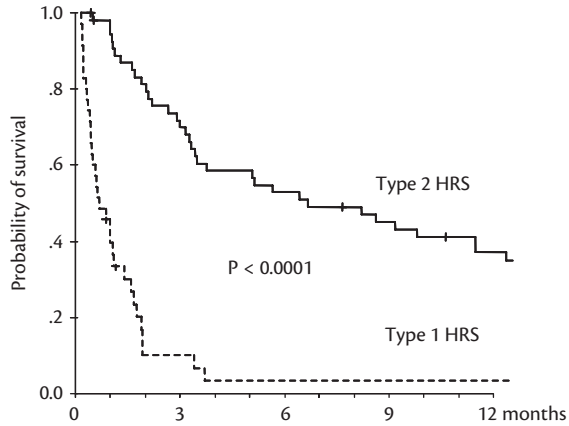

Fig. 169.4 Probability of survival of patients with cirrhosis according to type of hepatorenal syndrome (HRS).
Reproduced with permission from Alessandria, C., Ozdogan, O., Guevara, M., *et al.* (2005). MELD score and clinical type predict prognosis in hepatorenal syndrome: relevance to liver transplantation. *Hepatology*, 41, 1282–9.

with cirrhosis, sepsis, and AI treatment with hydrocortisone was associated with a rapid improvement in systemic haemodynamics and a reduction of vasopressor requirements (Fernández et al., 2006). More studies are needed to clarify the clinical importance of AI in cirrhosis and define the impact of corticosteroid supplementation in patients with liver disease and HRS.

Due to the lack of specific diagnostic markers, the diagnosis of HRS is made using accepted criteria that exclude other causes of renal failure that can occur in cirrhosis (Table 169.3) (Salerno et al., 2007). There are two types of HRS: in type 1, HRS renal function deteriorates rapidly with an increase in serum creatinine to a level > 2.5 mg/dL in < 2 weeks. This type of HRS is associated with a very poor prognosis without treatment with a median survival time of only 2 weeks if untreated (Ginès et al., 1993; Alessandria et al., 2005) (Fig. 169.4). In type 2 HRS, there is a steady impairment of renal function and serum creatinine levels usually range between 1.5 and 2.5 mg/dL. Patients with type 2 HRS have a median survival time of 6 months if not transplanted (Fig. 169.4). Patients with type 2 HRS may go on to develop type 1 HRS, either due to progression of disease or triggering factors such as bacterial infections. Although the differential diagnosis between HRS and ATN remains difficult and the presence of granular casts may be observed in the urine sediments of both HRS and ATN, if renal tubular cells are seen this favours the diagnosis of ATN (Ginès and Schrier, 2009).

Drug-induced renal failure

Drug nephrotoxicity in patients with cirrhosis may arise in patients taking diuretics, NSAIDs, antihypertensives, or drugs that can cause ATN, and/or acute interstitial nephritis (AIN) (Salerno and Badalamenti, 2005). Haemodynamically mediated renal failure is mainly caused by NSAIDs. These drugs alter the equilibrium between vasodilator and vasoconstrictor factors in the renal circulation. NSAIDs inhibit the enzymes cycloxygenase-1 and 2 which are responsible for prostaglandin synthesis. As described above, prostaglandins are important renal vasodilators that contribute significantly in maintaining normal renal perfusion. The risk of developing renal failure due to NSAID administration is higher in patients with cirrhosis and ascites and increased activity of the vasoconstrictor systems (Brater et al., 1986). Renal failure after NSAID use is followed by a rapid improvement of the GFR to pre-treatment values after cessation of the drug in most cases. Diuretic-induced renal failure is usually moderate and reversible after diuretic withdrawal and is related to an imbalance between the fluid loss from the intravascular space caused by diuretic treatment and the passage of fluid from the peritoneal compartment to the general circulation. Drug-induced ATN occurs mainly due to the use of aminoglycosides, amphotericin B, or vancomycin. Other drugs used in patients with chronic liver disease undergoing therapy for hepatitis B that may cause renal toxicity are the antivirals adefovir and tenofovir. The specific mechanisms that lead to this toxicity are not completely understood. Thus, it must be taken into account that patients with cirrhosis and renal failure being treated may need to switch to an alternative antiviral such as lamivudine or entecavir. Finally, AIN may occur due to antibiotics such as penicillin, rifampin, and sulphonamides as well as with NSAIDs, proton pump inhibitors, or allopurinol. In drug-induced AIN, there is an inflammatory component that affects the renal tubules and interstitium and which occurs as a hypersensitivity reaction to medications. In most cases, renal failure will return to normal function after discontinuation of the offending agent.

Management

General measures

Successful management of patients with cirrhosis and renal failure depends on the prompt recognition of renal failure and of its underlying cause. Patients with severe AKI should be admitted to a monitored unit where an appropriate workup and therapy can be performed. If there is any suspicion of an associated bacterial infection, in most cases third-generation cephalosporins are the initial treatment of choice while awaiting cultures (European Association for the Study of the Liver, 2010). Nonetheless, a very detailed history of past hospital admissions should be sought after because nosocomial infections and bacterial resistance render, in many cases, third-generation cephalosporins ineffective in these patients. Several multiresistant bacteria, especially extended spectrum beta-lactamase-producing *Enterobacteriaceae*, are commonly found in nosocomial and even healthcare-related infections, thus appropriate and alternate antibiotic coverage should be considered for these patients (Fernández et al., 2012). Patients with renal failure and severe sepsis may have associated relative AI and may benefit from hydrocortisone administration; however, more studies are needed in this area before recommending steroids in patients with advanced cirrhosis (Fede et al., 2012b). Patients with renal failure and hypovolaemia usually respond to volume repletion (saline or colloids solutions) and management of GI bleeding if present. Most patients with drug-induced renal disease will have improved renal function upon discontinuation of the toxic drug. Patients with AKI that require therapy for large-volume ascites or oedema should not be treated with diuretics. These patients benefit from large-volume paracentesis and administration of albumin (8 g/L of ascites removed) if necessary (European Association for the Study of the Liver, 2010). Although cirrhotic patients rarely develop renal failure after contrast media for radiological studies, they should undergo standard prophylactic measures such as saline hydration and monitoring of renal function after the procedure. The main objective of patients with HRS, particularly those

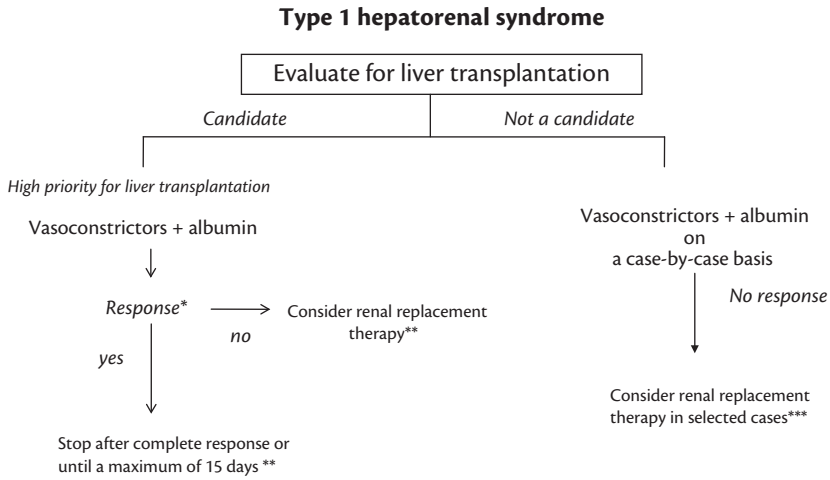

Fig. 169.5 Management strategy for patients with type-1 hepatorenal syndrome. * Response to therapy with vasoconstrictors plus albumin is considered when there is a reduction of serum creatinine to a level below 1.5 mg/dL. ** If there is recurrence, patients should be re-treated. *** Renal replacement therapy should be reserved for patients with severe volume overload, intractable acidosis or severe hyperkalaemia.

awaiting LT, is reversing renal failure in order to provide a successful bridge to transplantation. The best available therapy for HRS other than LT is the use of splanchnic vasoconstrictors plus albumin. Other modalities such as a transjugular intrahepatic portosystemic shunt (TIPS), renal replacement therapy (RRT), and albumin dialysis may be useful in some patients, but data on these approaches is limited (Fig. 169.5).

Vasoconstrictors

The administration of vasoconstrictors is the best medical therapy currently available for the management of HRS. The rationale of this therapy is to improve circulatory function by causing vasoconstriction of the extremely dilated splanchnic arterial bed, which subsequently improves arterial underfilling, reduces the activity of the endogenous vasoconstrictor systems, and increases renal perfusion. The available vasoconstrictors used in HRS are vasopressin analogues (terlipressin) and alpha-adrenergic agonists (noradrenaline (norepinephrine) or midodrine), which act on V1 vasopressin receptors and α_1 adrenergic receptors, respectively, present in vascular smooth muscle cells (Table 169.4). In most studies, vasoconstrictors have been given in combination with IV albumin to further improve the arterial underfilling (Table 169.4). Most of the published data comes from the use of IV terlipressin for type 1 HRS (Uriz et al., 2000; Moreau, et al., 2002; Ortega et al., 2001; Halimi et al., 2002; Solanki et al., 2003; Alessandria et al., 2007; Neri et al., 2008; Sanyal et al., 2008; Martin-Llahi et al., 2008; Triantos et al., 2010; Gluud et al, 2010; Sagi et al., 2010; Narahara et al., 2012). Initial non-controlled studies showed a response rate of 60–75%. However, results from recent randomized controlled studies and systematic reviews indicate that treatment with terlipressin together with albumin is associated with a marked response rate of approximately 40–50% (Martin-Llahi et al., 2008; Sanyal et al., 2008; Gluud et al., 2010; Sagi et al., 2010).

Although there are no dose-efficacy studies, treatment is typically started with 1 mg/4–6 hours IV, and the dose is increased up to a maximum of 2 mg/4–6 hours after 3 days if there is no response to therapy as defined by a reduction of serum creatinine > 25% of pre-treatment values. Response to therapy is considered when there is marked reduction of the high serum creatinine levels, at least below 1.5 mg/dL, which is usually associated with increased urine output and improvement of hyponatraemia. The incidence of side effects requiring the discontinuation of treatment is of approximately 7% (Sagi et al., 2010). Two randomized studies described previously (Martin-Llahi et al., 2008; Sanyal et al., 2008) demonstrated that the overall population of patients treated with terlipressin and albumin do not have an improved survival compared to that of patients treated with albumin alone. Nonetheless, these studies had a low sample size which could have hindered a survival benefit of treatment. In any case, both studies showed that responders in terms of improvement of renal function after therapy had an improved survival compared to non-responders. Recurrence of HRS after withdrawal of therapy occurs in < 10% of patients and retreatment with terlipressin is generally effective (Sagi et al., 2010). Factors associated with poor

Table 169.4 Pharmacological treatment of hepatorenal syndrome

Vasoconstrictors	*Terlipressin:* 1 mg/4–6 h IV; the dose is increased up to a maximum of 2 mg/4–6 h after 3 days if there is no response to therapy as defined by a reduction of serum creatinine > 25% of pre-treatment values Response to therapy is considered when there is marked reduction of the high serum creatinine levels, at least below 1.5 mg/dL (133 µmol/L). Treatment is usually given from 5 to 15 days
	Midodrine and octreotide: midodrine 7.5 mg orally three times daily, increased to 12.5 mg three times daily if needed. Octreotide 100 micrograms subcutaneously three times daily, increased to 200 micrograms three times daily if needed
	Noradrenaline (norepinephrine): 0.5–3 mg/h as continuous IV infusion aimed at increasing mean arterial pressure by 10 mmHg. Treatment is maintained until serum creatinine decreases below 1.5 mg/dL
Albumin administration	Concomitant administration of albumin together with vasoconstrictor drugs (1 g/kg body weight at day 1 followed by 20–40 g/day)

response include a bilirubin level ≥ 10 mg/dL, no increase in mean arterial pressure > 5 mmHg, or lack of a drop in serum creatinine > 0.5mg/dL at day 3 of therapy (Nazar et al., 2010). In addition another study showed that patients with baseline serum creatinine < 5.6 mg/dL and receiving > 3 days of therapy achieved HRS reversal (Boyer et al., 2011). Finally a systematic review of studies concluded that an increase in mean arterial pressure during vasoconstrictor therapy in patients with HRS was strongly associated with a decrease in serum creatinine which suggests that tailoring therapy to increase the baseline mean arterial pressure is beneficial for these patients (Velez and Nietert, 2011). This indicates that less severe renal failure (i.e. lower serum creatinine) and an increase in mean arterial pressure during therapy are associated with a good probability of HRS reversal in those patients treated with terlipressin.

Alpha-adrenergic agonists (noradrenaline (norepinephrine), midodrine) represent an attractive alternative to terlipressin because of their low cost and wide availability (Angeli et al., 1999; Duvoux et al., 2002; Wong et al., 2004; Alessandria et al., 2007; Skagen et al., 2009) (Table 169.4). However, the information on the efficacy and side effects of alpha-adrenergic agonists in patients with type 1 HRS is still limited. A recent controlled study that compared IV terlipressin and albumin versus octreotide/midodrine plus albumin in patients with type 1 HRS (Cavallin et al., 2015) showed that improvement of renal function was significantly better in patients receiving terlipressin and albumin (70.4%) than in patients treated with midodrine and octreotide and albumin (28.6%). This trial is the first that specifically compared both treatment regimens and the results indicate that terlipressin is superior to octreotide and midodrine and thus a preferred treatment option for patients with type 1 HRS.

There is limited data on use of vasoconstrictors plus albumin for patients with type 2 HRS. However, data from uncontrolled studies suggest that they are effective in decreasing serum creatinine levels in these patients. In two controlled studies, patients with type 2 HRS who received terlipressin plus albumin had a response between 67% and 88%, however few were treated with this strategy in both studies (N = 13) and therefore more studies are needed in order to better define the role of vasoconstrictors plus albumin in the management of type 2 HRS (Alessandria et al., 2007; Martin-Llahi et al., 2008).

Transjugular intrahepatic portosystemic shunts

The use of portosystemic shunts for therapy of HRS has been suggested for years, but the applicability in patients with such advanced liver disease is very limited. Two small studies indicate that TIPS may improve GFR as well as reduce the activity of the renin–angiotensin–aldosterone system and the sympathetic nervous system in patients with type 1 HRS (Guevara et al., 1998; Brensing et al., 2000). Although these studies included patients with advanced liver failure, they excluded those with a history of hepatic encephalopathy, Child–Pugh scores ≥ 12, or serum bilirubin > 5 mg/dL. Additionally, the applicability of TIPS in patients with type 1 HRS is low because TIPS is considered contraindicated in patients with features of severe liver failure and high MELD (model of end-stage liver disease) scores, which are common findings in the setting of type 1 HRS. The use of TIPS in type 2 HRS may improve renal function and reduce the risk of progression to type 1 HRS, but these data would require confirmation in specifically designed studies (Ginès et al., 2002).

Renal replacement therapy and other dialysis methods

RRT, mainly haemodialysis, has been used in the management of patients with type 1 HRS, especially in patients who are candidates for LT, in an attempt to maintain patients alive until LT is performed or spontaneous improvement in renal function occurs (Gonwa and Wadei, 2012). Unfortunately, the potential beneficial effect of this approach has not been demonstrated in randomized studies comparing RRT with other forms of therapy such as vasoconstrictors. Most patients develop side effects during RRT which include severe arterial hypotension, bleeding, and infections that may contribute to death during treatment. Additionally, indications for RRT (severe fluid overload, acidosis, or hyperkalaemia) are uncommon in type 1 HRS, at least in the early stages. Other methods such as the use of the molecular readsorbent recirculating system (MARS), an alternative of dialysis that clears albumin-bound substances, including vasodilators, is promising but more data are needed in order to consider it as a therapeutic device for HRS (Bañares et al., 2010). The results of a recent study using a fractionated plasma separation and adsorption extracorporeal liver support system, Prometheus, suggest that this technique improve survival in patients with type 1 HRS and acute-on-chronic liver failure; however, these results require confirmation in larger studies (Kribben et al., 2012).

Liver transplantation

LT is the treatment of choice for patients with cirrhosis and HRS. Unfortunately a drawback of LT as a treatment modality for type 1 HRS is the very high mortality rates many patients exhibit in the waiting list. This limitation is usually overcome by assigning these patients a high priority for transplantation. Since pre-transplant renal failure is an independent risk factor of both short-term and long-term post-transplantation patient and graft survival, all efforts should be made to improve renal function in order to obtain a better outcome after transplantation (Charlton et al., 2009). The reversal of both type 1 and 2 HRS before transplantation may help patients not only reach transplantation, but also reduce the relatively high morbidity after LT characteristic of HRS. An analysis of a cohort of patients with type 1 HRS treated with terlipressin and albumin versus albumin alone revealed that among those receiving terlipressin plus albumin, the 6-month survival rates were 100% for patients that underwent LT (Boyer et al., 2011). Interestingly, the group receiving only albumin and who underwent LT had a survival rate of 94% at 6 months. The survival rate was significantly better (47%) for those achieving a reversal of HRS versus those not achieving a reversal (4%). In the analysis, terlipressin did not confer a significant post-transplant survival benefit because the 6-month survival rate for both groups was similar. What is clear is that LT is beneficial for patients with type 1 HRS regardless of the type of treatment they receive. The advantage of using terlipressin in patients undergoing LT relates mainly to its effect of improving renal function which makes taking care of the patients easier while awaiting LT and, most likely, during the post-transplant period. More studies and a longer follow-up period are still needed to determine whether pre-LT therapy of HRS actually will translate into better post-LT outcomes.

Prevention

HRS can be prevented in some clinical settings. In the patients with SBP, the risk of HRS is substantial, but it may be prevented with

the administration of IV albumin (1.5 g/kg at diagnosis of infection and 1 g/kg 48 hours later). This measure prevents worsening of circulatory dysfunction and the subsequent development of HRS. The rationale for albumin administration is to prevent further arterial underfilling and subsequent activation of vasoconstrictor systems, which usually occur in the setting of SBP in patients with cirrhosis (Sort et al., 1999). The incidence of HRS in patients with SBP who receive albumin together with antibiotic therapy is 10%, compared with an incidence of 33% in those not receiving albumin (Sort et al., 1999). More importantly, there is improved survival in those receiving albumin (10%) versus those not receiving albumin (29%). In patients with very advanced liver disease, the long-term administration of norfloxacin orally (400 mg/day) prevents the development of HRS. In patients with ascitic fluid containing < 15 g of proteins/L (1.5 g/dL) and associated liver and/or renal function impairment (bilirubin > 3 mg/dL, a Child–Pugh score > 10, serum sodium < 130 mmol/L, and/or serum creatinine > 1.2 mg/dL), norfloxacin reduces the risk of HRS and improves survival (Fernández et al., 2007). The reason why norfloxacin prevents this complication is likely related to its ability to prevent bacterial translocation, suppress proinflammatory cytokines, and improve circulatory function (Chin-Dusting et al., 1997; Rasaratnam et al., 2003). Finally, a large study showed that in the setting of acute alcoholic hepatitis, the use of *N*-acetylcysteine for 5 days plus prednisolone prevented death due to HRS. Mortality at 6 months was higher in the prednisolone-only group compared with the *N*-acetylcysteine group (22% vs 9%) (Nguyen-Khac et al., 2011).

References

Alessandria, C., Ottobrelli, A., Debernardi-Venon, W., *et al.* (2007). Noradrenalin vs terlipressin in patients with hepatorenal syndrome: a prospective, randomized, unblinded, pilot study. *J Hepatol*, 47, 499–505.

Alessandria, C., Ozdogan, O., Guevara, M., *et al.* (2005). MELD score and clinical type predict prognosis in hepatorenal syndrome: relevance to liver transplantation. *Hepatology*, 41, 1282–9.

Angeli P., Gines P., Wong F., *et al.* (2015). Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. *J Hepatol*, 62(4), 968–74.

Angeli, P., Volpin, R., Gerunda, G., *et al.* (1999). Reversal of type 1 hepatorenal syndrome with the administration of midodrine and octreotide. *Hepatology*, 29, 1690–97.

Arroyo, V. and Fernández, J. (2011). Management of hepatorenal syndrome in patients with cirrhosis. *Nat Rev Nephrol*, 7, 517–26.

Arroyo, V., Ginès, P., Gerbes, A. L., *et al.* (1996). Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International Ascites Club. *Hepatology*, 23, 164–76.

Arvaniti, V., D'Amico, G., Fede, G., *et al.* (2010). Infections in patients with cirrhosis increase mortality 4-fold and should be used in determining prognosis. *Gastroenterology*, 139, 1246–56.

Bañares, R., Nevens, F., Larsen, F. S., *et al.* (2010). Extracorporeal liver support with the molecular adsorbent recirculating system (MARS) in patients with acute-on-chronic liver failure (AOCLF). The RELIEF Trial. *J Hepatol*, 52, 1184A.

Brater, D. C., Anderson, S. A., Brown-Cartwright, D., *et al.* (1986). Effects of nonsteroidal antiinflammatory drugs on renal function in patients with renal insufficiency and in cirrhotics. *Am J Kidney* Dis, 8, 351–5.

Brensing, K. A., Textor, J., Perz, J., *et al.* (2000). Long term outcome after transjugular intrahepatic portosystemic stent-shunt in non-transplant cirrhotics with hepatorenal syndrome: a phase II study. *Gut*, 47, 288–95.

Boyer, T. D., Sanyal, A. J., Garcia-Tsao, G., *et al.* (2011). Predictors of response to terlipressin plus albumin in hepatorenal syndrome (HRS) type 1: relationship of serum creatinine to hemodynamics. *J Hepatol*, 55, 315–21.

Boyer, T. D., Sanyal, A. J., Garcia-Tsao, G., *et al.* (2011). Impact of liver transplantation on the survival of patients treated for hepatorenal syndrome type 1. *Liver Transpl*, 17, 1328–32.

Cárdenas, A., Ginès, P., Uriz, J., *et al.* (2001). Renal failure after upper gastrointestinal bleeding in cirrhosis: incidence, clinical course, predictive factors, and short-term prognosis. *Hepatology*, 34, 671–6.

Caregaro, L., Menon, F., Angeli, P., *et al.* (1994). Limitations of serum creatinine level and creatinine clearance as filtration markers in cirrhosis. *Arch Intern Med*, 154, 201–5.

Cavallin, M., Kamath, P. S., Merli, M., *et al.* (2015). Terlipressin plus albumin versus midodrine and octreotide plus albumin in the treatment of hepatorenal syndrome: a randomized trial. *Hepatology*, doi: 10.1002/hep.27709.

Charlton, M. R., Wall, W. J., Ojo, A. O., *et al.* (2009). Report of the first International Liver Transplantation Society expert panel consensus conference on renal insufficiency after liver transplantation. *Liver Transpl*, 2009 15 , S1–S34.

Chin-Dusting, J. P., Rasaratnam, B., Jennings, G. L., *et al.* (1997). Effect of fluoroquinolone on the enhanced nitric oxide-induced peripheral vasodilation seen in cirrhosis. *Ann Intern Med*, 127, 985–8.

Cockcroft, D. W. and Gault, M. H. (1976). Prediction of creatinine clearance from serum creatinine. *Nephron*, 16, 31–41.

Duvoux, C., Zanditenas, D., Hezode, C., *et al.* (2002). Effects of noradrenaline and albumin in patients with type 1 hepatorenal syndrome: a pilot study. *Hepatology*, 36, 374–80.

European Association for the Study of the Liver (2010). EASL Clinical Practice Guidelines. Management of ascites, spontaneous bacterial peritonitis and hepatorenal syndrome in cirrhosis. *J Hepatol*, 53, 397–417.

Fasolato, S., Angeli, P., Dallagnese, L., *et al.* (2007). Renal failure and bacterial infections in patients with cirrhosis: epidemiology and clinical features. *Hepatology*, 45, 223–9.

Fede, G., D'Amico, G., Arvaniti, V., *et al.* (2012a). Renal failure and cirrhosis: a systematic review of mortality and prognosis. *J Hepatol*, 56, 810–18.

Fede, G., Spadaro, L., Tomaselli, T., *et al.* (2012b). Adrenocortical dysfunction in liver disease: A systematic review. *Hepatology*, 55, 1282–91.

Fernández, J., Escorsell, A., Zabalza, M., *et al.* (2006). Adrenal insufficiency in patients with cirrosis and septic shock: Effect of treatment with hydrocortisone on survival. *Hepatology*, 44, 1288–95

Fernández, J., Navasa, M., Gómez, J., *et al.* (2002). Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology*, 35, 140–8.

Fernández, J., Navasa, M., Planas, R., *et al.* (2007). Primary prophylaxis of spontaneous bacterial peritonitis delays hepatorenal syndrome and improves survival in cirrhosis. *Gastroenterology*, 133, 818–24.

Fernández, J., Acevedo, J., Castro, M., *et al.* (2012). Prevalence and risk factors of infections by multiresistant bacteria in cirrhosis: a prospective study. *Hepatology*, 55, 1551–61.

Francoz, C., Glotz, D., Moreau, R., *et al.* (2011). The evaluation of renal function and disease in patients with cirrhosis. *J Hepatol*, 52, 605–13.

Francoz, C., Prié, D., Abdelrazek, W., *et al.* (2010). Inaccuracies of creatinine and creatinine-based equations in candidates for liver transplantation with low creatinine: impact on the model for end-stage liver disease score. *Liver Transpl*, 16, 1169–77.

Ginès, A., Escorsell, A., Ginès, P., *et al.* (1993). Incidence, predictive factors, and prognosis of the hepatorenal syndrome in cirrhosis with ascites. *Gastroenterology*, 105, 229–36.

Ginès, P., Cárdenas, A., and Schrier, R. W. (2007). Liver disease and the kidney. In R. W. Schrier (ed.) *Diseases of the Kidney and Urinary Tract* (8th ed.), pp. 2179–205. Philadelphia, PA: Lippincott Wilkins and Williams.

Ginès, P. and Schrier, R. W. (2009). Renal failure in cirrhosis. *N Engl J Med*, 361, 1279–90.

Ginès, P., Uriz, J., Calahorra, B., *et al.* (2002). Transjugular intrahepatic portosystemic shunting versus paracentesis plus albumin for refractory ascites in cirrhosis. *Gastroenterology*, 123, 1839–47.

Gluud, L. L., Christensen, K., Christensen, E., *et al.* (2010). Systematic review of randomized trials on vasoconstrictor drugs for hepatorenal syndrome. *Hepatology*, 51, 576–84.
Gonwa, T. A., Jennings, L., Mai, M. L., *et al.* (2004). Estimation of glomerular filtration rates before and after orthotopic liver transplantation: evaluation of current equations. *Liver Transplant*, 10, 301–9.
Gonwa, T. A. and Wadei, H. M. (2012). The challenges of providing renal replacement therapy in decompensated liver cirrhosis. *Blood Purif*, 33, 144–8.
Guevara, M., Ginès, P., Bandi, J. C., *et al.* (1998). Transjugular intrahepatic portosystemic shunt in hepatorenal syndrome: effects on renal function and vasoactive systems. *Hepatology*, 28, 416–22.
Halimi, C., Bonnard, P., and Bernard, B. (2002). Effect of terlipressin (Glypressin) on hepatorenal syndrome in cirrhotic patients: results of a multicentre pilot study. *Eur J Gastroenterol Hepatol*, 14, 153–8.
Hecker, R. and Sherlock, S. (1956). Electrolyte and circulatory changes in terminal liver failure. *Lancet*, 271, 1121–5.
Krag, A., Bendtsen, F., Henriksen, J. H., *et al.* (2010). Low cardiac output predicts development of hepatorenal syndrome and survival in patients with cirrhosis and ascites. *Gut*, 59, 105–10.
Kribben, A., Gerken, G., Haag, S., *et al.* (2012). Effects of fractionated plasma separation and adsorption on survival in patients with acute-on-chronic liver failure. *Gastroenterology*, 142, 782–89.
Lancestremere, R. G., Davidson, P. L., Earley, L. E., *et al.* (1962). Renal failure in Laennec's cirrhosis. II. Simultaneous determination of cardiac output and renal hemodynamics. *J Clin Invest*, 41, 1922–7.
Lhotta, K. (2002). Beyond hepatorenal syndrome: glomerulonephritis in patients with liver disease. *Semin Nephrol*, 22, 302–8.
Martin-Llahi, M., Guevara, M., Torre, A., *et al.* (2011). Prognostic importance of the cause of renal failure in patients with cirrhosis. *Gastroenterology*, 140, 488–96.
Martin-Llahi, M., Pepin, M. N., Guevara, G., *et al.* (2008). Terlipressin and albumin vs albumin in patients with cirrhosis and hepatorenal syndrome: a randomized study. *Gastroenterology*, 134, 1352–9.
Montoliu, S., Ballesté, B., Planas, R., *et al.* (2010). Incidence and prognosis of different types of functional renal failure in cirrhotic patients with ascites. *Clin Gastroenterol Hepatol*, 8, 616–22.
Moreau, R., Durand, F., Poynard, T., *et al.* (2002). Terlipressin in patients with cirrhosis and type 1 hepatorenal syndrome: a retrospective multicenter study. *Gastroenterology*, 122, 923–30.
Narahara, Y., Kanazawa, H., Sakamoto, C., *et al.* (2012). The efficacy and safety of terlipressin and albumin in patients with type 1 hepatorenal syndrome: a multicenter, open-label, explorative study. *J Gastroenterol*, 47(3), 313–20.
Nazar, A., Pereira, G. H., Guevara, M., *et al.* (2010). Predictors of response to therapy with terlipressin and albumin in patients with cirrhosis and type 1 hepatorenal syndrome. *Hepatology*, 51, 219–26.
Neri, S., Pulvirenti, D., Malaguarnera, M., *et al.* (2008). Terlipressin and albumin in patients with cirrhosis and type I hepatorenal syndrome. *Dig Dis Sci*, 53, 830–5.
Nguyen-Khac, E., Thevenot, T., Piquet, M. A., *et al.* (2011). Glucocorticoids plus N-acetylcysteine in severe alcoholic hepatitis. *N Engl J Med*, 365, 1781–9.
Ortega, R., Ginès, P., Uriz, J., *et al.* (2002). Terlipressin therapy with and without albumin for patients with hepatorenal syndrome. Efficacy and outcome. *Hepatology*, 36, 941–8.
Papadakis, M. A. and Arieff, A. I. (1987). Unpredictability of clinical evaluation of renal function in cirrhosis. Prospective study. *Am J Med*, 82, 945–52.
Proulx, N. L., Akbari, A., Garg, A. X., *et al.* (2005). Measured creatinine clearance from timed urine collections substantially overestimates glomerular filtration rate in patients with liver cirrhosis: a systematic review and individual patient meta-analysis. *Nephrol Dial Transplant*, 20, 1617–22.
Rasaratnam, B., Kaye, D., Jennings, G., *et al.* (2003). The effect of selective intestinal decontamination on the hyperdynamic circulatory state in cirrhosis. A randomized trial. *Ann Intern Med*, 139(3), 186–93.
Ruiz-del-Arbol, L., Urman, J., Fernández, J., *et al.* (2003). Systemic, renal, and hepatic hemodynamic derangement in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology*, 38, 1210–18.
Ruiz-del-Arbol, L., Monescillo, A., Arocena, C., *et al.* (2005). Circulatory function and hepatorenal syndrome in cirrhosis. *Hepatology*, 42, 439–47.
Sagi, S. V., Mittal, S., Kasturi, K. S., *et al.* (2010). Terlipressin therapy for reversal of type 1 hepatorenal syndrome: a meta-analysis of randomized controlled trials. *J Gastroenterol Hepatol*, 25, 880–5.
Salerno, F. and Badalamenti, S. (2005). Drug induced renal failure in cirrhosis. In P. Ginès, V. Arroyo, J. Rodes, *et al.* (eds.) *Ascites and Renal Dysfunction in Liver Disease* (2nd ed.), pp. 372–82. Oxford: Blackwell Publishing.
Salerno, F., Gerbes, A., Wong, F., *et al.* (2007). Diagnosis, prevention and treatment of the hepatorenal syndrome in cirrhosis. A consensus workshop of the international ascites club. *Gut*, 56, 1310–18.
Sanyal, A., Boyer, T., Garcia-Tsao, G., *et al.* (2008). A prospective, randomized, double blind, placebo-controlled trial of terlipressin for type 1 hepatorenal syndrome (HRS). *Gastroenterology*, 134, 1360–8.
Schrier, R. W., Arroyo, V., Bernardi, M., *et al.* (1988). Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology*, 8, 1151–7.
Schroeder, E. T., Shear, L., Sancetta, S. M., *et al.* (1967). Renal failure in patients with cirrhosis of liver. 3. Evaluation of intrarenal blood flow by para-aminohippurate extraction and response to angiotensin. *Am J Med*, 43(6), 887–96.
Shear, L., Kleinerman, J., and Gabuzda, G. J. (1965). Renal failure in patients with cirrhosis of liver. I. Clinical and pathologic characteristics. *Am J Med*, 39(2), 184–96.
Skagen, C., Einstein, M., Lucey, M. R., *et al.* (2009). Combination treatment with octreotide, midodrine, and albumin improves survival in patients with type 1 and type 2 hepatorenal syndrome. *J Clin Gastroenterol*, 43, 680–5.
Solanki, P., Chawla, A., Garg, R., *et al.* (2003). Beneficial effects of terlipressin in hepatorenal syndrome: a prospective, randomized placebo-controlled clinical trial. *J Gastroenterol Hepatol*, 18, 152–6.
Sort, P., Navasa, M., Arroyo, V., *et al.* (1999). Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med*, 341, 403–9.
Tandon, P. and Garcia-Tsao, G. (2008). Bacterial infections, sepsis, and multiorgan failure in cirrhosis. *Semin Liver Dis*, 28, 26–42.
Trawalé, J. M., Paradis, V., Rautou, P. E., *et al.* (2010). The spectrum of renal lesions in patients with cirrhosis: a clinicopathological study. *Liver Int*, 30, 725–32.
Triantos, C. K., Samonakis, D., Thalheimer, U., *et al.* (2010). Terlipressin therapy for renal failure in cirrhosis. *Eur J Gastroenterol Hepatol*, 22, 481–6.
Tsai, M. H., Peng, Y. S., Chen, Y. C., *et al.* (2006). Adrenal insufficiency in patients with cirrhosis, severe sepsis and septic shock. *Hepatology*, 43, 673–81.
Uriz, J., Ginès, P., Cardenas, A., *et al.* (2000). Terlipressin plus albumin infusion: an effective and safe therapy of hepatorenal syndrome. *J Hepatol*, 33, 43–8.
Velez, J. C. and Nietert, P. J. (2011). Therapeutic response to vasoconstrictors in hepatorenal syndrome parallels increase in mean arterial pressure: a pooled analysis of clinical trials. *Am J Kidney Dis*, 58(6), 928–38.
Wadei, H. M., Geiger, X. J., Cortese, C., *et al.* (2008). Kidney allocation to liver transplant candidates with renal failure of undetermined etiology: role of percutaneous renal biopsy. *Am J Transplant*, 8, 2618–26.
Wong, F., Pantea, L., and Sniderman, K. (2004). Midodrine, octreotide, albumin, and TIPS in selected patients with cirrhosis and type 1 hepatorenal syndrome. *Hepatology*, 40, 55–64.
Wong, L. P., Blackley, M. P., Andreoni, K. A., *et al.* (2005). Survival of liver transplant candidates with acute renal failure receiving renal replacement therapy. *Kidney Int*, 68, 362–70.
Wong, F., Nadim, M. K., Kellum, J. A., *et al.* (2011). Working Party proposal for a revised classification system of renal dysfunction in patients with cirrhosis. *Gut*, 60, 702–9.

CHAPTER 170

Kidney/ear syndromes

Paul Goodyer

Introduction

The human embryo is only about 0.5 cm long at the time organogenesis begins at 3–4 weeks' gestation. This involves a complex pattern of cell movements and the expression of genes or micro-RNAs that initiate differentiation cascades. The mammalian kidney develops from progenitor cells in the intermediate mesoderm, while components of the external, middle, and inner ear arise from the surface ectoderm and foregut endoderm. Why is it that some children are born with disturbances of these two organs that appear to be formed so independently? In some instances it may be explained by mutation of genes that are part of molecular developmental pathways shared by the kidneys and inner ear. It may also reflect mutation of genes orchestrating shared physiologic pathways involving an epithelial layer and the fluid in which it is bathed. At times it may involve shared sensitivity to environmental influences such as toxic drugs. In any case, the interesting group of kidney/ear syndromes is the subject of this chapter.

Development of the kidney

In the early embryo, bilateral nephric ducts begin their descent towards the cloaca and express a discrete set of transcription factors, *PAX2, PAX8*, and *GATA3* (Grote et al., 2006; and see Chapter 343). Flanking each nephric duct are longitudinal columns of intermediate mesoderm containing quiescent progenitor cells that express transcription factors, including *OSR1 EYA1, PAX2, SIX1*, and *SALL1*, that organize secretion of glial cell-derived neurotrophic factor (GDNF) (Costantini and Kopan, 2010) (Fig. 170.1A). At the appropriate level along the body axis, GDNF levels are sufficient to elicit outgrowth of a ureteric bud from each nephric duct. As the ureteric buds penetrate the intermediate mesoderm, they begin to arborize and, at each branch tip, secrete molecular signals that induce the adjacent renal progenitor cells to cluster and express another wave of transcription factors (e.g. *SIX2* and *SALL1*) (Fig. 170.1B). The mesenchymal progenitor cells undergo dramatic transformation to form polarized epithelia lining a 'renal vesicle'. This new vesicular structure twists into an S-shape (S), fusing at its distal end with the ureteric bud while capillary ingrowth forms the glomerulus at its proximal end. As the first nephrons are differentiating, the ureteric bud grows outwards, branches again, and induces another generation of nephrons from the renal progenitor pool. In humans, this process is reiterated 18–20 times before nephrogenesis finally ends at 36 weeks' gestation. At birth, nephron endowment is set for life and each kidney contains about 600,000–800,000 nephrons (Nyengaard and Bendtsen, 1992).

Development of the ear

Development of the human ear begins by the fourth week of gestation, when paired plates of surface ectoderm invaginate to form the otic pits. These pinch off from the surface to form the left and right otic vesicles (Wu and Kelley, 2012). In parallel, the external ear develops from surface ectoderm around the first branchial groove while the middle ear (tympanic cavity) is initiated from foregut endoderm. Cartilaginous centres from the first pharyngeal pouch are incorporated into the tympanic cavity and these will later ossify to form the ossicles that transmit sound waves to the inner ear.

In the second month of gestation, the otic vesicle divides into three parts: (a) the endolymphatic ducts and saccule, (b) a central expanded utricle, and (c) the cochlear duct (Fig. 170.2). The endolymphatic ducts form semicircular canals and the expanded utricle is lined by sensory hair cells that contact the vestibular branch of the eighth cranial nerve. Thus, mechanostimulation of stereocilia by fluid movement of endolymph produces afferent signals that respond to sudden movements of the head that are critical for balance. Meanwhile, the cochlear duct forms 2.5 spirals and gives rise to the organ of Corti innervated by the cochlear branch of the eighth cranial nerve. Sound waves, conducted mechanically from the ear drum to the round window, move through the cochlear endolymph to the tectorial membrane within the cochlear canal. A highly organized layer of inner ear hair cells contact the tectorial membrane and respond to movement by allowing an influx of potassium from the endolymph that activates acoustic nerve signals. Potassium-rich endolymph is secreted by the stria vascularis and is reabsorbed in a diverticulum of the central canal expressing a chloride/bicarbonate exchanger (pendrin).

As the inner ear develops, EYA1 initiates a molecular cascade involving SIX1 that is crucial for regional specification of sensory cells. The otic epithelium of homozygous mutant *Eya1* or *Six1* mice undergoes apoptosis and inner ear development is arrested (Zou et al., 2006). Normally, *PAX2* and *GATA3* are expressed in the otic vesicle and homozygous *Pax2* mutant mice often lack an endolymphatic duct and have only a rudimentary cochlea (Burton et al., 2004). *GATA3* is more widely expressed in the inner ear than *PAX2* (Lawoko-Kerali et al., 2002), but even heterozygous inactivation of *GATA3* causes progressive loss of cochlear hair cells.

Ear syndromes caused by mutant developmental genes

Mother Nature is parsimonious with developmental programmes that have taken a long time to evolve. Many such molecular cascades are shared by organs that, on the surface, appear to be very

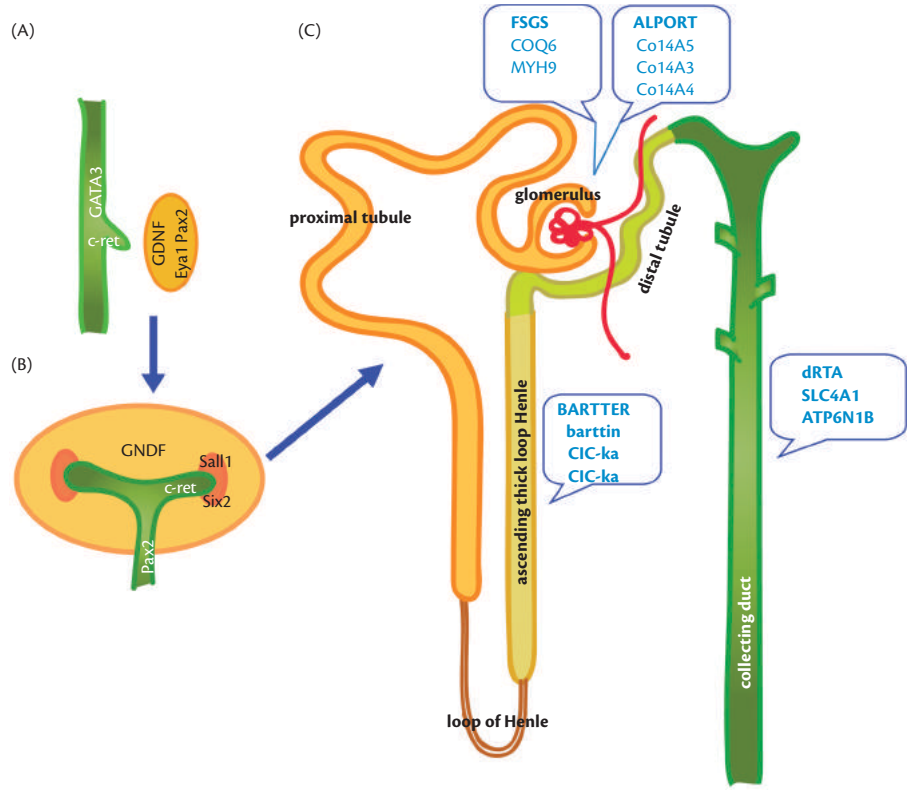

Fig. 170.1 Development of the kidney (see text and further detail in Chapter 343).

different. Although the kidney and ear have different embryologic origins and, in the end, serve very different functions, a number of kidney–ear syndromes represent the dysfunction of specific mutant genes that are required for development of both organs.

Branchio-oto-renal syndrome (OMIM 113650)

A syndrome of pre-auricular pits or branchial fistulae, deafness, and renal hypoplasia was noted in the nineteenth century, but the first detailed description of the autosomal dominant brancho-oto-renal (BOR) syndrome was in 1978 (Melnick et al., 1978) (see Chapter 358). Typical features include a mixed form of conductive/sensorineural hearing loss, with preauricular pits, a 'cup-shaped' deformity of ear pinnae, external auditory canal stenosis, branchial fistulae, and renal anomalies including primary renal hypoplasia, ureteropelvic junction obstruction, and vesicoureteral reflux. Renal hypoplasia ranges from slightly reduced kidney size to bilateral renal agenesis with perinatal death (Chitayat et al., 1992) and may affect one kidney more than the other. Additional minor anomalies occurring in < 20% of BOR families were delineated (Chang et al., 2004). The prevalence of BOR syndrome is about 1:40,000 and affects 2% of children with profound deafness (Orten et al., 2008). The cochlea is hypoplastic and may be simplified; dilatation of the vestibular duct may be evident by magnetic resonance imaging (Chang et al., 2004). Renal hypoplasia is highly variable

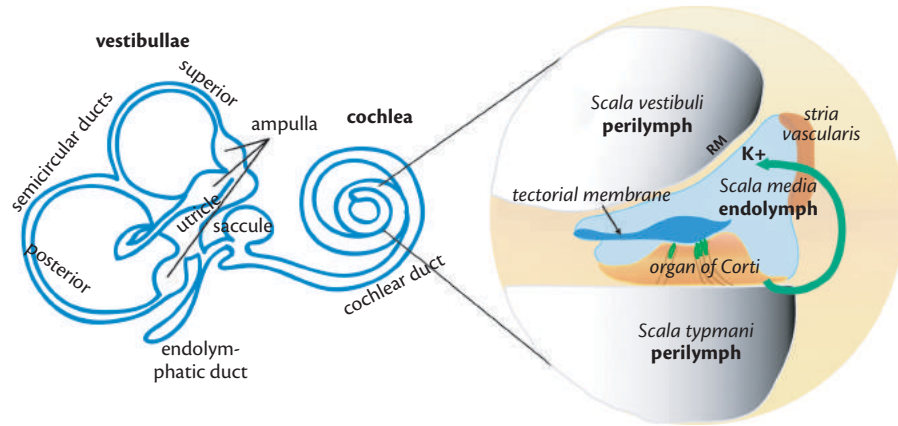

Fig. 170.2 Development of the ear (see text for explanation).

and only 5–10% of BOR patients develop end-stage renal disease (ESRD) (Orten et al., 2008). A patient support group may be found online at <http://www.thecainfoundation.com>.

About 40% of BOR syndrome patients have heterozygous inactivating mutations of the transcription factor *EYA1* gene (8q13) (Abdelhak et al., 1997). Over 80 different mutations of *EYA1* have been reported (Orten et al., 2008). *EYA1* is expressed in mesenchymal progenitor cells of the kidney and first branchial arch that gives rise to the auricles, auditory canal and ossicles of the ear; *EYA1* is expressed in hair cells of the cochlea (Kalatzis et al., 1998). Thus *EYA1* mutations cause renal hypoplasia, outer ear deformities, and conductive hearing loss.

Between 5% and 10% of BOR syndrome patients have mutations of genes belonging to the SIX transcription factor family. Several families bear mutations of *SIX1* (Ruf et al., 2004) and nearly 5% have mutations of *SIX5* (Hoskins et al., 2007). SIX family genes participate in the EYA1 complex that regulates GDNF expression in progenitor cells (Costantini and Kopan, 2010). Although it has not yet been implicated in BOR syndrome, SIX2 is also crucial for progenitor cells prior to nephrogenesis (Park et al., 2012) and is expressed in the otic placode (Ghanbari et al., 2001). Thus, BOR syndrome is heterogeneous and can be caused by a variety of genetic lesions interfering with the EYA/SIX pathway which sets early cell fate in metanephric mesenchyme and in branchial arches linked to the inner ear.

Renal-coloboma syndrome (OMIM 120330)

Since the 1970s, paediatric nephrologists have recognized a rare autosomal dominant form of 'oligomeganephronia' in which renal hypoplasia and progressive proteinuric renal failure in childhood are associated with large glomeruli and optic nerve colobomas (Carter and Lirenman, 1970) (see Chapter 360). Young children with this renal-coloboma syndrome (RCS; also known as papillorenal syndrome) have proteinuria and hypertension, but progressive renal failure is variable and ESRD may be delayed until adulthood (Iatropoulos et al., 2012). RCS is caused by heterozygous mutations of the *PAX2* gene; in some families there is associated vesico-ureteral reflux and high-frequency hearing loss (Eccles and Schimmenti, 1999). About 60 different *PAX2* mutations have been described, the most common being a single base-pair insertion in the second exon (Porteous et al., 2000). In the heterozygous *Pax2* mutant mouse, loss of PAX2 increases apoptosis of ureteric bud cells and compromises ureteric bud arborization reducing congenital nephron number (Porteous et al., 2000). *Pax2* is expressed in the developing inner ear where it interacts with EYA1 to regulate cell fate (Freter et al., 2012). A human *PAX2* mutation registry (<https://grenada.lumc.nl/LOVD2/PAX2/home.php>) has been established.

Hypoparathyroidism, sensorineural deafness, and renal disease syndrome (OMIM 146255)

Barakat et al. described two brothers with sensorineural deafness, hypoparathyroidism in infancy, steroid-resistant nephrotic syndrome, and ESRD by the end of the first decade (Barakat et al., 1977). Bilous et al. recognized hypoparathyroidism, sensorineural deafness, and renal disease (HDR) as an autosomal dominant syndrome in 1992 (Bilous et al., 1992). About 8 years later, it was shown that the syndrome is caused by heterozygous mutations of the *GATA3* gene on chromosome 10p14 (Van Esch et al., 2000). Affected patients exhibit congenital hypocalcaemia with undetectable serum levels of parathyroid hormone and moderately severe high-frequency sensorineural deafness, requiring early use of hearing aids. Women also have malformation of the uterus and vagina. Renal dysplasia may lead to ESRD but is variable, ranging from renal agenesis to normal-sized kidneys associated with vesicoureteral reflux. A summary of the disease is available via the Orphanet website: <http://www.orpha.net/consor/cgi>.

GATA3 belongs to a family of zinc-finger transcription factors and is expressed in the nephric duct, parathyroid glands, and inner ear during early gestation (Labastie et al., 1995). During embryogenesis, it is crucial for rostrocaudal descent of the nephric duct. Mice with homozygous *Gata3* knockout develop multiple misguided nephric ducts that fail to induce metanephric kidneys (Grote et al., 2006). During development of the inner ear, *GATA3* is expressed in cochlear hair cells; mice bearing one null *Gata3* allele have hearing loss (van Looij et al., 2006). GATA3 seems to function as a survival factor both in nephric duct and in hair cells.

Townes–Brocks syndrome (OMIM 107480)

In 1972, Townes and Brocks described a patient with imperforate anus, sensorineural deafness, minor anomalies of the external ear (pre-auricular tags and deformity of the superior helix), and multiple anomalies of the digits including missing bones and supernumerary thumbs (Townes and Brocks, 1972). Clinical features of the Townes–Brocks syndrome (TBS) (see Chapter 359) syndrome are now recognized to be more complex and include renal hypoplasia/dysplasia (Sudo et al., 2010). About half of TBS patients have heterozygous mutations of the transcription factor gene *SALL1* which can sometimes lead to ESRD; dominant-negative *SALL1* mutations produce a slightly more severe disease (Faguer et al., 2009). A family support group is available at <http://health.groups.yahoo.com/group/Townes-Brocks-Syndrome>.

In developing mouse kidneys, *SALL1* is expressed in the metanephric mesenchyme surrounding branches of the ureteric bud; *Sall1* knockout mice lack outgrowth of the ureteric bud and die perinatally of renal agenesis (Nishinakamura and Osafune, 2006). Recent studies suggest a role for Sall1 in progenitor cells of the kidney (Harrison et al., 2012), but the role of SALL1 in the developing ear has not been studied in detail. TBS patients have combined sensorineural and conductive hearing deficits associated with dysplasia of the ossicles of the middle ear and oval window of the cochlea (Izzedine et al., 2004). By inference, SALL1 must play some role in development of the middle ear from mesenchymal cells of the first branchial arch.

Ciliopathies and kidney/ear syndromes

In recent years, the non-motile primary cilium projecting from the surface of most cells has been 'rediscovered' by the biomedical community (Hildebrandt et al., 2011) (see Chapter 303). The cilium serves as a nexus for key intracellular signalling pathways that govern cell biology. Dysfunction of cilia on renal tubular cells is associated with various renal cystic diseases. Cilia also decorate the surface of hair cells in the organ of Corti and vestibular apparatus where they are essential for inner ear function (Wu and Kelley, 2012).

Bardet–Biedl syndrome (OMIM 209900)

Bardet–Biedl syndrome (BBS) (see Chapter 314) is a rare (1:50,000) multi-organ dysplasia characterized by obesity, rod-cone dystrophy,

mental retardation, polydactyly and hypogenitalism in association with various renal anomalies and deafness. Other features include diabetes, anosmia, situs inversus, and heart defects. BBS is genetically heterogeneous: about 80% of cases are linked to mutation of 18 genes associated with the primary cilium, basal body, or centrosomes (Forsythe and Beales, 2013). Renal anomalies are variable, ranging from complete renal agenesis (rare) to cystic dysplasia. Among 20 BBS patients from Newfoundland, half had developed hypertension but only 15% had developed ESRD by 31 years of age (Harnett et al., 1988). Retinal dystrophy is a cardinal feature of BBS and seems to reflect the central role of cilial signalling in the eye. Overt deafness is not usually listed as an important feature of BBS, despite studies implicating cilia in the physiology of inner ear hair cells. However, formal assessment identified hearing deficits at 1 kHz and 4 kHz in 53% and 84% of BBS patients (Ross et al., 2005; Billingsley et al., 2010).

Alstrom syndrome (OMIM 203800)

In 1959, Alstrom described an autosomal recessive syndrome of blindness, profound hearing loss, type 2 diabetes, and obesity in children (Alstrom et al., 1959) (see Chapter 314). Sensorineural deafness progresses gradually and is severe in 10%. A recent re-evaluation showed renal insufficiency in half of the 182 patients between 5 and 42 years of age (Marshall et al., 2007). This was associated with widespread interstitial fibrosis on renal biopsy. The syndrome is caused by mutations in the *ALMS1* gene encoding a protein localized at the cilial basal body (Hearn et al., 2002). Mice with an insertional mutation in *Alsm1* have enlarged kidneys, dilation of proximal tubules, and interstitial inflammation, but a mechanism linking the putative cilial function of ALMS1 to deafness and renal dysfunction is not yet established (Collin et al., 2005).

Genes required for specialized physiology in the kidney and ear

During terminal differentiation, cells express genes for specialized proteins that carry out organ-specific tasks. While the function of the ear and kidney are quite different, nature often utilizes the same tools in different ways. Along the renal tubules, secretory and reabsorptive epithelial transporters modify the makeup of tubular fluid to achieve whole-body electrolyte homeostasis. Similarly, epithelial cells of the inner ear regulate the makeup of endolymph to facilitate hearing. One subset of kidney–ear syndromes reflect shared physiologic processes in the mature organ. (See Fig. 170.1C.)

Distal renal tubular acidosis with deafness (OMIM 267300)

In distal renal tubular acidosis (dRTA) (see Chapter 36), alpha-intercalated cells of the collecting duct are unable to secrete the daily metabolic acid load (about 2 mEq/kg/day). Infants with inherited forms of dRTA fail to thrive because of persistent acidosis from birth. In some families, the trait is restricted to the kidney (Batlle and Haque, 2012) as in (a) autosomal dominant dRTA due to heterozygous mutations of the chloride/bicarbonate exchanger gene *SLC4A1* (Karet et al., 1998) and (b) autosomal recessive dRTA caused by homozygous mutations of the *ATP6N1B* gene, encoding the kidney-specific B subunit of the apical proton pump of intercalated cells (Smith et al., 2000). However, there are two additional forms of recessive dRTA in which patients also develop deafness. These involve mutant genes encoding the B1 and A4 subunits of the apical proton pump in kidney and cochlea (Karet et al., 1999; Stover et al., 2002). Loss of these subunits blocks urinary acidification but also compromises acidity of endolymph bathing cochlear hair cells. Patients with homozygous mutations of *ATP6V1B1* have dRTA from birth and develop deafness with aqueduct enlargement detectable by computed tomography (CT) scan during early childhood (3 months to 2 years) (Nikki et al., 2012). Despite nephrocalcinosis, these patients do not typically develop renal failure as adults (Feldman et al., 2006). Patients with mutations of the A4 subunit gene (*ATP6V0A4*) are more common (about two-thirds of cases) but have milder, more variable deafness developing anywhere between early childhood and young adulthood (Vargas-Poussou et al., 2006). A patient registry is maintained by Orphanet at <http://www.orpha.net>.

Bartter syndrome with deafness (OMIM 602522)

In Bartter syndrome (see Chapter 31), defective salt reabsorption in the thick ascending limb of the loop of Henle (TALH) causes massive polyuria, volume contraction, hyper-reninaemia, metabolic alkalosis, and failure to thrive. Normally, the TALH reabsorbs 15–30% of filtered salt via the apical sodium–potassium–chloride cotransporter (NKCC2); potassium is recycled via the apical ROMK channel to the tubular lumen where it primes another round of cotransport. Sodium entering the cell is actively pumped across the basolateral membrane by Na/K-ATPase while chloride diffuses down its concentration gradient through two basolateral chloride channels, ClC-Ka and ClC-Kb. Both chloride channels must bind to a common protein (barttin) for proper targeting to the basolateral membrane.

Neonatal Bartter syndrome may involve homozygous mutations of the genes for NKCC2 or ROMK, but these channels are kidney specific and the ear is unaffected. In contrast, barttin and ClC-Ka are co-expressed in the thin limb of Henle's loop and in stria vascularis cells of the inner ear (Uchida et al., 1995; Hayama et al., 2003). Infants born with homozygous mutations of the barttin gene (*BSND*) (Estevez et al., 2001) or digenic mutations of the two chloride channel genes (Schlingmann et al., 2004) display profound renal salt wasting and congenital deafness. These patients present with maternal polyhydramnios, premature birth, and severe electrolyte disturbances (hyponatraemia, hypokalaemia, and metabolic alkalosis) in the newborn period. Deafness is apparent within the first year of life and massive salt wasting stimulates renin secretion to levels 20–25 times the normal range (Shalev et al., 2003). Salt wasting is reduced by indomethacin therapy (2 mg/kg/day). In some (but not all) cases, ESRD associated with tubulointerstitial fibrosis develops in childhood (Park et al., 2011). Referral to cochlear implant centres improves speech perception and development. Information about Bartter syndrome with deafness is found at <http://www.RareRenal.org>.

Barttin and ClC-Kb are co-expressed in the loop of Henle, distal convoluted tubule, and intercalated cells of the collecting duct as well as in cells of the stria vascularis and vestibular apparatus of the inner ear (Kobayashi et al., 2002). In response to vibrations conducted along the scala media, endolymph potassium normally enters outer hair cells via mechanosensitive channels. Potassium then cycles out of the hair cell, is taken up by a KCl co-transporter into adjacent Deiter cells, and is then transported via gap junctions to the stria vascularis. Cells of the stria vascularis make indirect

use of barttin-activated chloride channels to pump potassium back into the endolymph. Unlike the kidney, cochlear hair cells use the $Na/K/Cl_2$ co-transporter to take up electrolytes from the basolateral surface and then recycle sodium via the basolateral Na/K-ATPase, accompanied by passive chloride efflux through the bartin-activated ClC-Ka and ClC-Kb channels. The only path for efflux of potassium is into the endolymph via KCNQ1 channels at the apical surface.

Alport syndrome (OMIM 301050, 203780)

Alport syndrome (see Chapter 321) is a hereditary progressive nephropathy associated with deformity of the ocular lens, retinal disease, and high-tone sensorineural deafness due to loss of cross-linking type IV collagens which are crucial for basement membrane assembly (Kruegel et al., 2013). Alport syndrome affects about 1:5000 people worldwide and causes about 2% of ESRD in adults. Nearly 85% of cases are due to inactivating mutations of the *COL4A5* gene on the X chromosome (Kashtan, 2007). Autosomal recessive mutations of *COL4A3* and *COL4A4* genes on chromosome 2 account for < 10–15% of cases and a few families are affected by dominant negative missense mutations in these genes (Pescucci et al., 2004). In families where the inheritance pattern is unclear, diagnosis can now be made efficiently by next generation sequencing of all three genes (Artuso et al., 2012).

Affected males present in childhood with microscopic haematuria and patchy segments of thickened glomerular basement membrane. Loss of type IV collagen cross-linking weakens the α3/α4/α5 collagen network of the lamina densa which must contend with the pulsatile stretch of the glomerular capillary while providing a secure substratum for podocytes. With time, the helical multicollagen strands are frayed, producing a 'basket-weave' appearance on electron micrographs. Onset of proteinuria reflects damage to the glomerular podocytes. ESRD occurs in > 90% of affected males by age 40; about 12% of females with X-linked Alport syndrome also develop proteinuria and progressive renal insufficiency (Kashtan, 2007). A retrospective analysis has shown that early intervention with angiotensin-converting enzyme inhibitors in proteinuric children dramatically slows the progression of renal dysfunction (Gross et al., 2012). Prospective studies are under way. Practice guidelines from experts in Australia and North America emphasize genetic testing for the diagnosis of Alport syndrome; the need to follow all members of a family with X-linked Alport syndrome; early treatment of males with X-linked Alport syndrome and individuals with autosomal recessive disease with renin–angiotensin system blockade; and discouraging the affected mothers of males with X-linked Alport syndrome from renal donation because of their own risk of kidney failure (Kashtan et al., 2013).

Hearing loss is not congenital but begins in nearly half of affected males by age 10 and in 85% during adulthood. Deafness also develops in some women with X-linked Alport syndrome (Rheault, 2012) and in recessive Alport syndrome (Zhang et al., 2012). Both *COL4A3* and *COL4A5* are expressed in the basilar membrane and the spiral ligament of the cochlea (Zehnder et al., 2005). Basement membrane abnormalities are associated with cellular infilling of the tunnel and extracellular spaces of the organ of Corti (Merchant et al., 2004). Thus, mutation of all Alport genes is thought to cause sensorineural hearing loss by compromising cochlear micromechanics. Thus far, there does not appear to be a therapeutic intervention that ameliorates progressive hearing loss in Alport syndrome. Updates on Alport syndrome are available through the Alport Syndrome Foundation (<http://www.alportsyndrome.org>). An Alport Syndrome Treatment and Outcomes Registry (ASTOR) has been established.

MYH9-related syndromes (OMIM 160775)

Mutations of the non-muscle myosin *MYH9* gene (2q11) (see Chapter 342) cause four autosomal dominant syndromes (Epstein, Fechtner, Sebastien, and May–Hegglin syndromes) which include high-frequency sensorineural deafness, glomerulopathy with progressive renal insufficiency, and structural anomalies of circulating platelets and leucocytes (Balduini et al., 2011). It is now thought that these four syndromes represent phenotypic variability of a single *MYH9*-related disease. *MYH9* encodes the heavy chain of a myosin that binds directly to actin filaments and seems to be involved in disassembly of the cytoskeleton during cell movement. It is expressed in the endothelium, various haematopoietic lineages and in a variety of tissues, including the kidney where it is expressed in both podocytes and tubules (Arrondel et al., 2002). The natural history of the disease was tracked in nine Japanese patients with heterozygous mutations of the R709 codon (Sekine et al., 2010). At presentation, most patients were thought to have idiopathic thrombocytopenia; platelets were large but reduced in number. Leucocytes showed granular inclusions (myosin heavy chain precipitates). The patients developed proteinuria and microscopic haematuria between 2 and 12 years of age with progressive renal insufficiency during adolescence, leading to ESRD between 15 and 20 years of age. In one case, serial renal biopsies showed only podocyte foot process effacement and focal glomerular basement membrane thickening by the end of the first decade. However, in the early teens, progressive renal insufficiency was heralded by the appearance of focal segmental glomerulosclerosis. Hearing impairment was evident before the age of 5 and all cases became completely deaf in early adulthood (Sekine et al., 2010). Early cochlear implantation may be important (Hildebrand et al., 2006).

COQ6 mutations and focal segmental glomerulosclerosis (OMIM514650)

In 2011, seven families with an autosomal recessive form of focal segmental glomerulosclerosis (FSGS) (see Chapter 327) and deafness (Heeringa et al., 2011) were found to have homozygous missense mutations in the gene (*COQ6*) encoding coenzyme Q_{10} biosynthesis mono-oxygenase. Index cases were drawn from Northern Lebanon and Turkey, where there is a founder mutation. A subsequent screen of 530 FSGS families identified two heterozygous *COQ6* missense mutations; in one of these individuals, proteinuria was partially responsive to ciclosporin therapy. However, none of the 11 individuals with homozygous mutations were responsive to immunosuppressive therapy (Heeringa et al., 2011). Patients with homozygous *COQ6* mutations typically present with proteinuria at about 1 year of age. Renal biopsies show FSGS at presentation and patients develop end-stage renal failure by the age of 3 years. Deafness was accompanied by neurologic symptoms including seizures and ataxia in some individuals (Heeringa et al., 2011).

COQ6 is required for synthesis of CoQ_{10}, a mitochondrial protein that participates in oxidative metabolism by shuttling electrons from early complexes I and II to complex III of the electron

transport chain. Interestingly, two children were treated with CoQ_{10} supplements (2 mg/day). In rats, they were able to show that the *COQ6* gene is expressed in podocytes and in the stria vascularis of the inner ear (Heeringa et al., 2011).

There are reports of patients with deafness and renal failure caused by mutations in other mitochondrial genes, including cytochrome b and tRNA(cys) (Feigenbaum et al., 2006). (See also Chapter 340.)

SeSAME/EAST syndrome (*KCJN10*) (OMIM 612780)

Mutations of the inwardly-rectifying potassium channel gene *KCNJ10* cause an autosomal recessive syndrome of epilepsy, ataxia, sensorineural deafness and renal tubulopathy (EAST) syndrome (Bockenhauer et al., 2009) (see Chapter 31). *KCNJ10* is expressed in the renal distal convoluted tubule and cortical TALH and in the stria vascularis of the cochlea (Reichold et al., 2010). Loss of *KCNJ10* in the kidney mimics the features of Gitelman syndrome, and causes hypokalaemic alkalosis, hypomagnesaemia, and hypocalciuria that gradually appear between 5 and 8 years of age (Scholl et al., 2012).

Kidney/ear syndromes caused by drug toxicity

Numerous therapeutic drugs cause damage to the kidney and ear, but gentamicin and cisplatin classically target these two organs. Although the mechanisms are not fully understood, the selective injury to proximal tubular cells of the kidney and marginal cells of the stria vascularis appears to depend on specific systems to take up and concentrate each drug in these tissues.

Gentamicin toxicity

Following therapeutic doses of gentamicin (see Chapter 362), 10–20% of patients exhibit a modest rise in serum creatinine and shed cellular and granular casts into the urinary sediment. Among cystic fibrosis patients who develop acute kidney injury, prior treatment with gentamicin therapy is a major risk factor (Smyth et al., 2008). Among adults treated with gentamicin for endocarditis, creatinine clearance falls by 0.5% for each day of therapy (Buchholtz et al., 2009). Two per cent of children who receive gentamicin transiently develop overt hearing loss (Echeverria et al., 1978). In adults, both hearing loss (17%) and vestibular dysfunction (30%) may be higher (Scheenstra et al., 2009). Among 103 adults with bilateral vestibular dysfunction following gentamicin (average of 3.5 mg/kg/day), only three had overt deafness (Ahmed et al., 2012). Although 20% first noted imbalance during gentamicin treatment, presentation was often delayed for years.

Recent studies with fluorescent-tagged drug indicate that gentamicin is selectively taken up via multivalent TRPV1 channels and concentrated by renal proximal tubular cells, cochlear hair cells and cells of the stria vascularis (Dai and Steyger, 2008). Following gentamicin treatment of newborns, significant increase in urinary excretion of the proximal tubular marker, kidney injury marker-1 (KIM-1), is evident for several days (McWilliam et al., 2012). In the ear, intracellular gentamicin generates free oxygen radicals which induce apoptotic cell death of inner ear hair cells.

In a study of 40 haemodialysis patients receiving 2 weeks of gentamicin therapy for catheter-related infections, administration of the antioxidant, *N*-acetylcysteine, significantly reduced ototoxicity assessed 1 and 6 weeks after antibiotic treatment (Feldman et al., 2007). Benefit from mitochondria-targeted antioxidants has been reported (Ojano-Dirain and Antonelli, 2012).

Some individuals are at especially high risk of aminoglycoside toxicity and may develop irreversible deafness after a single dose of gentamicin. These individuals belong to families (0.2% of Europeans) with maternal transmission of the mitochondrial DNA A1555G mutation which enhances gentamicin binding to the mitochondrial ribosome (Qian and Guan, 2009). Individuals carrying this A1555G mutation may develop deafness without drug exposure, but have > 95% risk of irreversible deafness if treated with gentamicin (Qian and Guan, 2009). It will be important to assess the effect of this mutation on nephrotoxicity in future studies.

Cisplatin toxicity in the kidney and ear

When cisplatin (see Chapter 362) chemotherapy was first introduced, nearly 70% of patients developed acute kidney injury (Launay-Vacher et al., 2008) and two-thirds also develop significant hearing loss (Dean et al., 2008). Although dose adjustment and intravenous hydration have improved tolerance to the drug, nephrotoxicity, and ototoxicity remain significant problems. In a study of 763 childhood cancer survivors studied 18 years later, estimated glomerular filtration rate was reduced by about 20% in those exposed to cisplatin (Dekkers et al., 2013).

Following a therapeutic dose of cisplatin, about 50% of the drug is excreted in the urine within 24 hours. However, it is specifically taken up by cells of the S3 segment of the renal proximal tubule and marginal cells of the stria vascularis and accumulates to levels many times the plasma concentration (Launay-Vacher et al., 2008). The uptake mechanism is unknown but does not involve the p-aminohippurate-inhibitable organic anion transport system. Once in the cell, cisplatin forms intrastrand cross-links between guanines of DNA in the nucleus and binds to a variety of cytoplasmic proteins; both events contribute to apoptotic cell death (Sheikh-Hamad, 2008). Within 48 hours of cisplatin, polyuria is noted, followed by a drop in glomerular filtration rate. Damage is not limited to the proximal tubule since magnesium wasting and distal renal tubular acidosis are prominent toxicities. In the ear, animal studies demonstrate selective damage to stria vascularis cells, fall of endocochlear potential, and loss of hearing within 24 hours of a high dose (16 mg/kg); repeated lower doses appear to produce toxicity in cochlear hair cells as well (Thomas et al., 2006).

References

Abdelhak, S., Kalatzis, V., Heilig, R., et al. (1997). Clustering of mutations responsible for branchio-oto-renal (BOR) syndrome in the eyes absent homologous region (eyaHR) of EYA1. *Hum Mol Genet*, 6, 2247–55.

Ahmed, R. M., Hannigan, I. P., MacDougall, H. G., *et al.* (2012). Gentamicin ototoxicity: a 23-year selected case series of 103 patients. *Med J Aust*, 196, 701–4.

Alstrom, C. H., Hallgren, B., Nilsson, L. B., *et al.* (1959). Retinal degeneration combined with obesity, diabetes mellitus and neurogenous deafness: a specific syndrome (not hitherto described) distinct from the Laurence-Moon-Bardet-Biedl syndrome: a clinical, endocrinological and genetic examination based on a large pedigree. *Acta Psychiatr Neurol Scand Suppl*, 129, 1–35.

Arrondel, C., Vodovar, N., Knebelmann, B., *et al.* (2002). Expression of the nonmuscle myosin heavy chain IIA in the human kidney and screening for MYH9 mutations in Epstein and Fechtner syndromes. *J Am Soc Nephrol*, 13, 65–74.

Artuso, R., Fallerini, C., Dosa, L., *et al.* (2012). Advances in Alport syndrome diagnosis using next-generation sequencing. *Eur J Hum Genet*, 20, 50–7.

Balduini, C. L., Pecci, A., and Savoia, A. (2011). Recent advances in the understanding and management of MYH9-related inherited thrombocytopenias. *Br J Haematol*, 154, 161–74.

Barakat, A. Y., D'Albora, J. B., Martin, M. M., *et al.* (1977). Familial nephrosis, nerve deafness, and hypoparathyroidism. *J Pediatr*, 91, 61–4.

Batlle, D. and Haque, S. K. (2012). Genetic causes and mechanisms of distal renal tubular acidosis. *Nephrol Dial Transplant*, 27, 3691–704.

Billingsley, G., Bin, J., Fieggen, K. J., *et al.* (2010). Mutations in chaperonin-like BBS genes are a major contributor to disease development in a multiethnic Bardet-Biedl syndrome patient population. *J Med Genet*, 47, 453–63.

Bilous, R. W., Murty, G., Parkinson, D. B., *et al.* (1992). Brief report: autosomal dominant familial hypoparathyroidism, sensorineural deafness, and renal dysplasia. *N Engl J Med*, 327, 1069–74.

Bockenhauer, D., Feather, S., Stanescu, H. C., *et al.* (2009). Epilepsy, ataxia, sensorineural deafness, tubulopathy, and KCNJ10 mutations. *N Engl J Med*, 360, 1960–70.

Buchholtz, K., Larsen, C. T., Hassager, C., *et al.* (2009). Severity of gentamicin's nephrotoxic effect on patients with infective endocarditis: a prospective observational cohort study of 373 patients. *Clin Infect Dis*, 48, 65–71.

Burton, Q., Cole, L. K., Mulheisen, M., *et al.* (2004). The role of Pax2 in mouse inner ear development. *Dev Biol*, 272, 161–75.

Carter, J. E. and Lirenman, D. S. (1970). Bilateral renal hypoplasia with oligomeganephronia. Oligomeganephronic renal hypoplasia. *Am J Dis Child*, 120, 537–42.

Chang, E. H., Menezes, M., Meyer, N. C., *et al.* (2004). Branchio-oto-renal syndrome: the mutation spectrum in EYA1 and its phenotypic consequences. *Hum Mutat*, 23, 582–9.

Chitayat, D., Hodgkinson, K. A., Chen, M. F., *et al.* (1992). Branchio-oto-renal syndrome: further delineation of an underdiagnosed syndrome. *Am J Med Genet*, 43, 970–5.

Collin, G. B., Cyr, E., Bronson, R., *et al.* (2005). Alms1-disrupted mice recapitulate human Alstrom syndrome. *Hum Mol Genet*, 14, 2323–33.

Costantini, F. and Kopan, R. (2010). Patterning a complex organ: branching morphogenesis and nephron segmentation in kidney development. *Dev Cell*, 18, 698–712.

Dai, C. F. and Steyger, P. S. (2008). A systemic gentamicin pathway across the stria vascularis. *Hear Res*, 235, 114–24.

Dean, J. B., Hayashi, S. S., Albert, C. M., *et al.* (2008). Hearing loss in pediatric oncology patients receiving carboplatin-containing regimens. *J Pediatr Hematol Oncol*, 30, 130–4.

Dekkers, I. A., Blijdorp, K., Cransberg, K., *et al.* (2013). Long-term nephrotoxicity in adult survivors of childhood cancer. *Clin J Am Soc Nephrol*, 8, 922–9.

Eccles, M. R., and Schimmenti, L. A. (1999). Renal-coloboma syndrome: a multi-system developmental disorder caused by PAX2 mutations. *Clin Genet*, 56, 1–9.

Echeverria, P., Fina, D., Norton, S., *et al.* (1978). Ototoxicity of gentamicin: clinical experience in a children's hospital. *Chemotherapy*, 24, 267–71.

Estevez, R., Boettger, T., Stein, V., *et al.* (2001). Barttin is a Cl- channel beta-subunit crucial for renal Cl- reabsorption and inner ear K+ secretion. *Nature*, 414, 558–61.

Faguer, S., Pillet, A., Chassaing, N., *et al.* (2009). Nephropathy in Townes-Brocks syndrome (SALL1 mutation): imaging and pathological findings in adulthood. *Nephrol Dial Transplant*, 24, 1341–5.

Feigenbaum, A., Bai, R. K., Doherty, E. S., *et al.* (2006). Novel mitochondrial DNA mutations associated with myopathy, cardiomyopathy, renal failure, and deafness. *Am J Med Genet A*, 140, 2216–22.

Feldman, L., Efrati, S., Eviatar, E., *et al.* (2007). Gentamicin-induced ototoxicity in hemodialysis patients is ameliorated by N-acetylcysteine. *Kidney Int*, 72, 359–63.

Feldman, M., Prikis, M., Athanasiou, Y., *et al.* (2006). Molecular investigation and long-term clinical progress in Greek Cypriot families with recessive distal renal tubular acidosis and sensorineural deafness due to mutations in the ATP6V1B1 gene. *Clin Genet*, 69, 135–44.

Forsythe, E., and Beales, P. L. (2013). Bardet-Biedl syndrome. *Eur J Hum Genet*, 21, 8–13.

Freter, S., Muta, Y., O'Neill, P., *et al.* (2012). Pax2 modulates proliferation during specification of the otic and epibranchial placodes. *Dev Dyn*, 241, 1716–28.

Ghanbari, H., Seo, H. C., Fjose, A., *et al.* (2001). Molecular cloning and embryonic expression of Xenopus Six homeobox genes. *Mech Dev*, 101, 271–7.

Gross, O., Licht, C., Anders, H. J., *et al.* (2012). Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. *Kidney Int*, 81, 494–501.

Grote, D., Souabni, A., Busslinger, M., *et al.* (2006). Pax 2/8-regulated Gata 3 expression is necessary for morphogenesis and guidance of the nephric duct in the developing kidney. *Development*, 133, 53–61.

Harnett, J. D., Green, J. S., Cramer, B. C., *et al.* (1988). The spectrum of renal disease in Laurence-Moon-Biedl syndrome. *N Engl J Med*, 319, 615–18.

Harrison, S. J., Nishinakamura, R., Jones, K. R., *et al.* (2012). Sall1 regulates cortical neurogenesis and laminar fate specification in mice: implications for neural abnormalities in Townes-Brocks syndrome. *Dis Model Mech*, 5, 351–65.

Hayama, A., Rai, T., Sasaki, S., *et al.* (2003). Molecular mechanisms of Bartter syndrome caused by mutations in the BSND gene. *Histochem Cell Biol*, 119, 485–93.

Hearn, T., Renforth, G. L., Spalluto, C., *et al.* (2002). Mutation of ALMS1, a large gene with a tandem repeat encoding 47 amino acids, causes Alstrom syndrome. *Nat Genet*, 31, 79–83.

Heeringa, S. F., Chernin, G., Chaki, M., *et al.* (2011). COQ6 mutations in human patients produce nephrotic syndrome with sensorineural deafness. *J Clin Invest*, 121, 2013–24.

Hildebrand, M. S., De Silva, M. G., Gardner, R. J., *et al.* (2006). Cochlear implants for DFNA17 deafness. *Laryngoscope*, 116, 2211–5.

Hildebrandt, F., Benzing, T., and Katsanis, N. (2011). Ciliopathies. *N Engl J Med*, 364, 1533–43.

Hoskins, B. E., Cramer, C. H., Silvius, D., *et al.* (2007). Transcription factor SIX5 is mutated in patients with branchio-oto-renal syndrome. *Am J Hum Genet*, 80, 800–4.

Iatropoulos, P., Daina, E., Mele, C., *et al.* (2012). Discordant phenotype in monozygotic twins with renal coloboma syndrome and a PAX2 mutation. *Pediatr Nephrol*, 27, 1989–93.

Izzedine, H., Tankere, F., Launay-Vacher, V., *et al.* (2004). Ear and kidney syndromes: molecular versus clinical approach. *Kidney Int*, 65, 369–85.

Kalatzis, V., Sahly, I., El-Amraoui, A., *et al.* (1998). Eya1 expression in the developing ear and kidney: towards the understanding of the pathogenesis of branchio-oto-renal (BOR) syndrome. *Dev Dyn*, 213, 486–99.

Karet, F. E., Finberg, K. E., Nelson, R. D., *et al.* (1999). Mutations in the gene encoding B1 subunit of H+-ATPase cause renal tubular acidosis with sensorineural deafness. *Nat Genet*, 21, 84–90.

Karet, F. E., Gainza, F. J., Gyory, A. Z., *et al.* (1998). Mutations in the chloride-bicarbonate exchanger gene AE1 cause autosomal dominant but not autosomal recessive distal renal tubular acidosis. *Proc Natl Acad Sci U S A*, 95, 6337–42.

Kashtan, C. E. (2007). Alport syndrome and the X chromosome: implications of a diagnosis of Alport syndrome in females. *Nephrol Dial Transplant*, 22, 1499–505.

Kashtan, C. E., Ding, J., Gregory, M., *et al.* (2013). Clinical practice recommendations for the treatment of Alport syndrome: a statement of the Alport Syndrome Research Collaborative. *Pediatr Nephrol*, 28, 5–11.

Kobayashi, K., Uchida, S., Okamura, H. O., *et al.* (2002). Human CLC-KB gene promoter drives the EGFP expression in the specific distal nephron segments and inner ear. *J Am Soc Nephrol*, 13, 1992–8.

Kruegel, J., Rubel, D., and Gross, O. (2013). Alport syndrome-insights from basic and clinical research. *Nat Rev Nephrol*, 9, 170–8.

Labastie, M. C., Catala, M., Gregoire, J. M., *et al.* (1995). The GATA-3 gene is expressed during human kidney embryogenesis. *Kidney Int*, 47, 1597–603.

Launay-Vacher, V., Rey, J. B., Isnard-Bagnis, C., *et al.* (2008). Prevention of cisplatin nephrotoxicity: state of the art and recommendations from the European Society of Clinical Pharmacy Special Interest Group on Cancer Care. *Cancer Chemother Pharmacol*, 61, 903–9.

Lawoko-Kerali, G., Rivolta, M. N., and Holley, M. (2002). Expression of the transcription factors GATA3 and Pax2 during development of the mammalian inner ear. *J Comp Neurol*, 442, 378–91.

Marshall, J. D., Beck, S., Maffei, P., *et al.* (2007). Alstrom syndrome. *Eur J Hum Genet*, 15, 1193–202.

McWilliam, S. J., Antoine, D. J., Sabbisetti, V., *et al.* (2012). Mechanism-based urinary biomarkers to identify the potential for aminoglycoside-induced nephrotoxicity in premature neonates: a proof-of-concept study. *PLoS One*, 7, e43809.

Melnick, M., Hodes, M. E., Nance, W. E., *et al.* (1978). Branchio-oto-renal dysplasia and branchio-oto dysplasia: two distinct autosomal dominant disorders. *Clin Genet*, 13, 425–42.

Merchant, S. N., Burgess, B. J., Adams, J. C., *et al.* (2004). Temporal bone histopathology in alport syndrome. *Laryngoscope*, 114, 1609–18.

Nikki, R., Martin, B., Gus, O., *et al.* (2012). Endolymphatic sac enlargement in a girl with a novel mutation for distal renal tubular acidosis and severe deafness. *Case Rep Pediatr*, 2012, 605053.

Nishinakamura, R., and Osafune, K. (2006). Essential roles of Sall family genes in kidney development. *J Physiol Sci*, 56, 131–6.

Nyengaard, J. R., and Bendtsen, T. F. (1992). Glomerular number and size in relation to age, kidney weight, and body surface in normal man. *Anat Rec*, 232, 194–201.

Ojano-Dirain, C. P., and Antonelli, P. J. (2012). Prevention of gentamicin-induced apoptosis with the mitochondria-targeted antioxidant mitoquinone. *Laryngoscope*, 122, 2543–8.

Orten, D. J., Fischer, S. M., Sorensen, J. L., *et al.* (2008). Branchio-oto-renal syndrome (BOR): novel mutations in the EYA1 gene, and a review of the mutational genetics of BOR. *Hum Mutat*, 29, 537–44.

Park, C. W., Lim, J. H., Youn, D. Y., *et al.* (2011). Renal dysfunction and barttin expression in Bartter syndrome Type IV associated with a G47R mutation in BSND in a family. *Clin Nephrol*, 75 Suppl 1, 69–74.

Park, J. S., Ma, W., O'Brien, L. L., *et al.* (2012). Six2 and Wnt regulate self-renewal and commitment of nephron progenitors through shared gene regulatory networks. *Dev Cell*, 23, 637–51.

Pescucci, C., Mari, F., Longo, I., *et al.* (2004). Autosomal-dominant Alport syndrome: natural history of a disease due to COL4A3 or COL4A4 gene. *Kidney Int*, 65, 1598–603.

Porteous, S., Torban, E., Cho, N. P., *et al.* (2000). Primary renal hypoplasia in humans and mice with PAX2 mutations: evidence of increased apoptosis in fetal kidneys of Pax2(1Neu) +/– mutant mice. *Hum Mol Genet*, 9, 1–11.

Qian, Y. and Guan, M. X. (2009). Interaction of aminoglycosides with human mitochondrial 12S rRNA carrying the deafness-associated mutation. *Antimicrob Agents Chemother*, 53, 4612–8.

Reichold, M., Zdebik, A. A., Lieberer, E., *et al.* (2010). KCNJ10 gene mutations causing EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) disrupt channel function. *Proc Natl Acad Sci U S A*, 107, 14490–5.

Rheault, M. N. (2012). Women and Alport syndrome. *Pediatr Nephrol*, 27, 41–6.

Ross, A. J., May-Simera, H., Eichers, E. R., *et al.* (2005). Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat Genet*, 37, 1135–40.

Ruf, R. G., Xu, P. X., Silvius, D., *et al.* (2004). SIX1 mutations cause branchio-oto-renal syndrome by disruption of EYA1-SIX1-DNA complexes. *Proc Natl Acad Sci U S A*, 101, 8090–5.

Scheenstra, R. J., Rijntjes, E., Tavy, D. L., *et al.* (2009). Vestibulotoxicity as a consequence of systemically administered tobramycin in cystic fibrosis patients. *Acta Otolaryngol*, 129, 4–7.

Schlingmann, K. P., Konrad, M., Jeck, N., *et al.* (2004). Salt wasting and deafness resulting from mutations in two chloride channels. *N Engl J Med*, 350, 1314–9.

Scholl, U. I., Dave, H. B., Lu, M., *et al.* (2012). SeSAME/EAST syndrome—phenotypic variability and delayed activity of the distal convoluted tubule. *Pediatr Nephrol*, 27, 2081–90.

Sekine, T., Konno, M., Sasaki, S., *et al.* (2010). Patients with Epstein-Fechtner syndromes owing to MYH9 R702 mutations develop progressive proteinuric renal disease. *Kidney Int*, 78, 207–14.

Shalev, H., Ohali, M., Kachko, L., *et al.* (2003). The neonatal variant of Bartter syndrome and deafness: preservation of renal function. *Pediatrics*, 112, 628–33.

Sheikh-Hamad, D. (2008). Cisplatin-induced cytoxicity: is the nucleus relevant? *Am J Physiol Renal Physiol*, 295, F42–3.

Smith, A. N., Skaug, J., Choate, K. A., *et al.* (2000). Mutations in ATP6N1B, encoding a new kidney vacuolar proton pump 116-kD subunit, cause recessive distal renal tubular acidosis with preserved hearing. *Nat Genet*, 26, 71–5.

Smyth, A., Lewis, S., Bertenshaw, C., *et al.* (2008). Case-control study of acute renal failure in patients with cystic fibrosis in the UK. *Thorax*, 63, 532–5.

Stover, E. H., Borthwick, K. J., Bavalia, C., *et al.* (2002). Novel ATP6V1B1 and ATP6V0A4 mutations in autosomal recessive distal renal tubular acidosis with new evidence for hearing loss. *J Med Genet*, 39, 796–803.

Sudo, Y., Numakura, C., Abe, A., *et al.* (2010). Phenotypic variability in a family with Townes-Brocks syndrome. *J Hum Genet*, 55, 550–1.

Thomas, J. P., Lautermann, J., Liedert, B., *et al.* (2006). High accumulation of platinum-DNA adducts in strial marginal cells of the cochlea is an early event in cisplatin but not carboplatin ototoxicity. *Mol Pharmacol*, 70, 23–9.

Townes, P. L. and Brocks, E. R. (1972). Hereditary syndrome of imperforate anus with hand, foot, and ear anomalies. *J Pediatr*, 81, 321–6.

Uchida, S., Sasaki, S., Nitta, K., *et al.* (1995). Localization and functional characterization of rat kidney-specific chloride channel, ClC-K1. *J Clin Invest*, 95, 104–13.

Van Esch, H., Groenen, P., Nesbit, M. A., *et al.* (2000). GATA3 haplo-insufficiency causes human HDR syndrome. *Nature*, 406, 419–22.

Van Looij, M. A., Meijers-Heijboer, H., Beetz, R., *et al.* (2006). Characteristics of hearing loss in HDR (hypoparathyroidism, sensorineural deafness, renal dysplasia) syndrome. *Audiol Neurootol*, 11, 373–9.

Vargas-Poussou, R., Houillier, P., Le Pottier, N., *et al.* (2006). Genetic investigation of autosomal recessive distal renal tubular acidosis: evidence for early sensorineural hearing loss associated with mutations in the ATP6V0A4 gene. *J Am Soc Nephrol*, 17, 1437–43.

Wu, D. K. and Kelley, M. W. (2012). Molecular mechanisms of inner ear development. *Cold Spring Harb Perspect Biol*, 4, a008409.

Zehnder, A. F., Adams, J. C., Santi, P. A., *et al.* (2005). Distribution of type IV collagen in the cochlea in Alport syndrome. *Arch Otolaryngol Head Neck Surg*, 131, 1007–13.

Zhang, Y., Wang, F., Ding, J., *et al.* (2012). Genotype-phenotype correlations in 17 Chinese patients with autosomal recessive Alport syndrome. *Am J Med Genet A*, 158A, 2188–93.

Zou, D., Silvius, D., Rodrigo-Blomqvist, S., *et al.* (2006). Eya1 regulates the growth of otic epithelium and interacts with Pax2 during the development of all sensory areas in the inner ear. *Dev Biol*, 298, 430–41.

CHAPTER 171

Kidney/eye syndromes

Friederike Mackensen, Vedat Schwenger, and Heiko Billing

Overview

The *eyes* are the *gateway* to the soul.

Herman Melville

How can findings in the eye prompt a diagnosis of a renal disease? And vice versa, how can the diagnosis of renal disease lead to an ophthalmologic consult to rule out eye disease?

A study on patients with chronic kidney disease revealed retinal pathologies in up to 25% of patients (Grunwald et al., 2010). Hypertensive and diabetic changes are by far the leading causes, but other entities as detailed in this chapter can also be found.

Of interest are the common pathways of kidney and eye disease. For example, type II membranous glomerulonephritis is characterized by the deposition of abnormal electron-dense material within the glomerular basement membrane of the kidney and within Bruch's membrane in the eye. The pathophysiologic basis is uncontrolled systemic activation of the alternative pathway of the complement cascade (Appel et al., 2005). In most patients, loss of complement regulation is caused by C3 nephritic factor, but in some patients, mutations in the factor H gene have been identified, a pathogenetic pathway also associated with one of the most frequent eye diseases, age-related macular degeneration (AMD) (Edwards et al., 2005; Klein et al., 2005). In Alport syndrome or hereditary nephritis, a number of patients show ocular abnormalities that are clearly related to defects in the basement membrane, but vary in frequency depending on gender and the genetic background (and thus the collagen chains affected) (Savige and Colville, 2009). One possible retinal manifestation is a dot-like retinopathy that is reminiscent of Drusen in dry AMD, but unlike this is not related to complement factor H (Liu et al., 2009). Recognizing the pathognomonic ocular changes can be helpful to lead to a diagnosis of Alport syndrome. Thus, retinal examination can be a highly sensitive and specific diagnostic test for Alport syndrome.

Another disease that affects kidney and eye is the tubulointerstitial nephritis and uveitis (TINU) syndrome. Genetic studies have revealed an association with human leucocyte antigen DRB0102 (Levinson et al., 2003), which could not be shown in the isolated nephritis (Mackensen et al., 2011). Other case reports have found autoantibodies against renal and retinal antigens in these patients (Wakaki et al., 2001; Abed et al., 2008). A hypersensitivity reaction that may be triggered by medications or a viral infection in a genetically susceptible individual is suspected. But why the immune system is activated to attack exactly these two organs at the same time remains unclear for the moment.

Abnormalities of the eye are common in patients on dialysis or after kidney transplantation and include (steroid-induced) cataract and opportunistic ocular infections. A transient cortical blindness can occur in severe uraemic states. On the other hand, ophthalmologists may indicate systemic immunosuppression, as ciclosporin after corneal transplantation may be harmful to renal function and thus requires monitoring by a nephrologist.

Another common interest between ophthalmologists and nephrologists in the near future may be to monitor treatment effects with the new imaging tools ophthalmology provides (e.g. *in vivo* confocal microscopy for corneal changes) (Guthoff et al., 2009).

To mimic the approach from eye findings to a diagnosis of renal disease, we decided to order this chapter following the anatomy of the eye, that is, the most frequently affected part of the eye. A summary is given in Table 171.1. If a specific ocular treatment modality exists, it will be mentioned at the end of each section.

Anterior segment changes

Adnexae

Congenital

- *Duane's anomaly* in *acro-renal syndrome*, less frequently microcornea, uveal and optic nerve coloboma. Duane's anomaly comprises a defect in ocular motility with a limitation of abduction with narrowing of the palpebral fissure and globe retraction on adduction.
- *Amyloidosis* is characterized by extracellular deposits of low-molecular-weight fibrils. Several variants, hereditary and acquired, are known. The kidney is one of the most common sites of amyloid deposition. The most frequent subtypes with renal involvement are the AL (as a consequence of immunoglobulin light chain disease) and the AA (due to ongoing inflammation) variants. Renal amyloid fibrils are predominantly deposited in the glomeruli, and therefore clinically associated with asymptomatic proteinuria. However, amyloid deposition

Table 171.1 Summary of diseases and syndromes that affect kidney and eye according to the part of the eye affected

	Amyloidosis	**Alport syn.**	**Cystinosis**	**Fabry disease**	**Acro-renal syn.**	**Nephrotic syn.**	**Vasculitis**	**Nephrogenic systemic fibrosis**	**LCAT-deficiency**	**Rathbun, WAGR-, Cat-Eye syn.**	**Pierson syn.**
Adnexae	Lid papules, purpura, oculomotor defects			Lesions	Duane´s anomaly	Lid oedema					
Conjunctiva	Nodules							Telangiectasia			
Sclera							Scleritis	yellow plaques			
Cornea	Dystrophy-like	Erosion, posterior dysmorphous dystrophy	Cystin crystals	Cornea verticillata	Microcornea				Opacity		Megalo-cornea, posterior embryo-toxon
Iris	Pupillary abnormalities			Nodules, hyphaema	Coloboma		(Uveitis)			Aniridia	Microcoria
Lens	Anterior lens capsule deposits	Anterior lenticonus, subcapsular cataract		Subcapsular cataract							Cataract
Vitreous	Opacities										Persistent vasculature
Retina	Deposits	Dot-and-fleck Retinopathy	crystals				Retinopathy				Detachment
Nerve	Neuropathy										

	Nail-patella syn.	**Oculocerebrorenal syn.**	**Von Hippel–Lindau disease**	**Galactosaemia**	**Oxalosis**	**Nephronophthisis, Bardet–Biedl, Alström syn.**	**Hypertension, diabetes**	**Type II glomerulonephritis**	**Papillorenal syn.**	**DIDMOAD**
Adnexae	Strabismus									
Conjunctiva										
Sclera										
Cornea	Keratoconus, microcornea									
Iris	pigmentation (Lester sign)									
Lens	microphacia, cataract	Bilateral cataract		Cataract						
Vitreous										
Retina			Haemangioblastoma		Flecked retinopathy	Retinitis pigmentosa like	Retinopathy	Drusen		
Nerve									Empty Disc	Atrophy

may be limited to vessels and then associated with renal failure rather than proteinuria. All parts of the eye have been reported to be affected by primary (AL) or familiar amyloidosis; secondary (AA) amyloidosis usually spares the eye (Brownstein et al., 1970). The more frequent effects are subconjunctival and periorbital haemorrhage and amyloid papules of lid or conjunctiva. Pupillary abnormalities and oculomotor dysfunction occur. Amyloid deposits in tear gland, cornea, lens, vitreous, and retina have been found.

- *Lid oedema* in *nephrotic syndrome*, which is caused by hypoalbuminaemia and consecutive decreased capillary oncotic pressure. Frequent causes of nephrotic syndrome are membranous glomerulonephritis, focal segmental glomerulosclerosis, minimal change disease, immunoglobulin A nephropathy, membranoproliferative glomerulonephritis, diabetic nephropathy and renal involvement in amyloidosis.

Conjunctiva

Acquired

- *Keratoconjunctivitis*: a typical and frequent sign of rheumatic diseases, that may also affect the kidney, is Sicca syndrome in Sjögren syndrome, rheumatoid arthritis, and systemic lupus. Patients complain about burning, foreign body sensation, and itching of the eyes. These symptoms significantly reduce patients' quality of life and they increase with computer work and/or reading.

Treatment

Generally lubricants, so-called artificial tears, of high viscosity and frequent application are helpful. In severe cases of conjunctivitis, short-term application of topical corticosteroids can be helpful; if needed, longer-term, topical 1–2% ciclosporin eye drops will be given. Punctum plugs, or permanent closure of the tear duct, are mechanical measures as are bandage contact lenses. In very severe cases, autologous serum eye drops or serum albumin eye drops are given.

Sclera

Acquired

- *Scleritis* is the most common eye involvement in antineutrophil cytoplasmic antibody-associated *vasculitides*. Granulomatosis with polyangiitis (Wegener granulomatosis) is the most common cause of scleritis in patients with systemic vasculitides. Uveitis has been seen anecdotally (Smith et al., 2007).
- *Nephrogenic systemic fibrosis* or nephrogenic systemic dermopathy which occurs not exclusively in dialysis patients, but in renal failure patients after exposure to gadolinium magnetic resonance imaging contrast agents: asymptomatic scleral involvement can be seen in as many as 75% of cases. Patients present with telangiectasia mimicking conjunctivitis and later yellow scleral plaques can be seen (Knopp and Cowper, 2008).

Cornea

Congenital

- *Amyloidosis: corneal dystrophy-like changes* can be observed which may be amyloid fibrils or immunoglobulin deposits and can lead to vision disturbance (Ruprecht and Naumann, 1997).
- *Cystinosis* is a genetic defect (caused by a *CTNS* gene mutation) of lysosomal transport of cystine leading to increased intralysosomal cystine accumulation in several organ systems. Cystine crystals in the cornea are seen as early as 1–2 years of age, a bronze-like picture, and lead to photophobia (see Fig. 171.1). It is an early symptom in 85% of the patients (Gahl et al., 2000). Retinal crystals are sometimes seen. Renal involvement presents as Fanconi syndrome and without a cystine-depleting therapy leads to terminal renal failure by the end of the first decade or even earlier.
- *Alport syndrome*: a primary basement membrane disorder arising from mutations in genes encoding several members of the type IV collagen protein family. Affected are basement membranes of the kidney (leading to progressive renal insufficiency), cochlea (causing hearing loss), and eye. Recurrent *corneal erosion* has been seen more frequently in patients with Alport syndrome than in controls and therefore in a young patient, should trigger suspicion of Alport syndrome (Rhys et al., 1997). Other ocular features include posterior dysmorphous corneal dystrophy, anterior lenticonus, and dot-and-fleck retinopathy (Colville and Savige, 1997). Renal symptoms begin early in childhood with persistent microscopic haematuria in male patients, regardless of

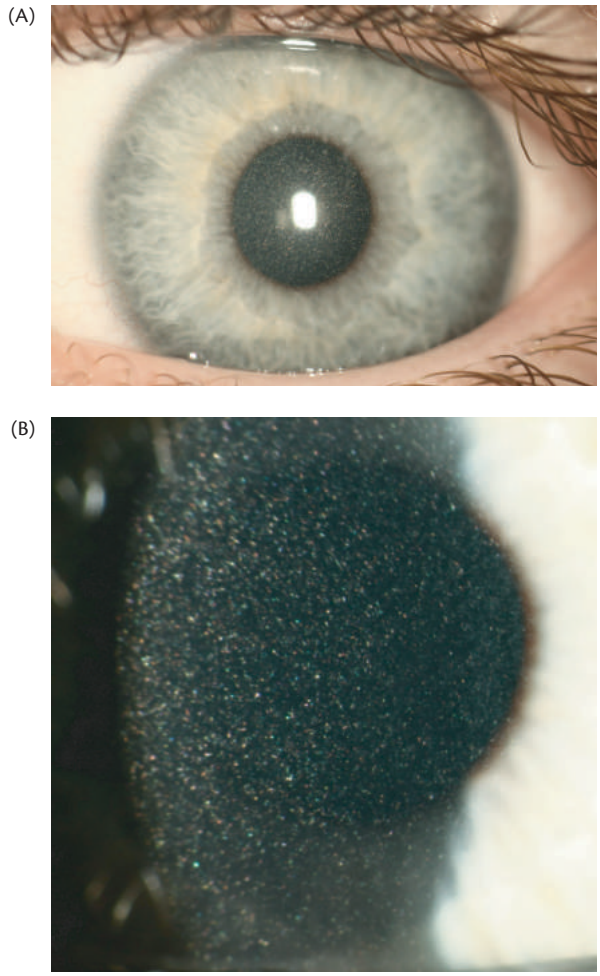

Fig. 171.1 (A) Slit-lamp photograph of an 8-year-old female with cystinosis. (B) Figure shows the light breaking cystine crystals in the cornea in larger magnification.

genetic type, whereas females with Alport syndrome may have persistent or intermittent haematuria (autosomal recessive or heterozygous X-linked Alport syndrome). Equally, the further course of developing end-stage renal disease (ESRD) is strongly gender dependent. ESRD may develop at age 25 years in 50% or even earlier (Jais et al., 2003).

- *Fabry disease*: eye involvement in almost all patients with Fabry disease includes whorl-like corneal deposits (*cornea verticillata*), which do not cause symptoms or compromise visual function. To see these deposits, slit-lamp examination may be necessary. They are similar to changes caused by systemic drugs such as amiodarone or chloroquine, but can be distinguished by appearance and history. Corneal lesions may be used to monitor treatment effect. Further lesions of the eye lids, iris nodules, recurring hyphaema and a subcapsular cataract can be seen (Sodi et al., 2006). Renal involvement in Fabry disease is common and increases with age. All patients develop ESRD over time. The renal pathology reveals accumulation of a degradation product of glycosphingolipid mainly in podocytes and tubulus cells. Renal pathological changes such as tubulointerstitial fibrosis and glomerular damage are closely associated with the residual alpha-Gal A activity.
- *Lecithin:cholesterol acyl transferase (LCAT) deficiency* (focal glomerulosclerosis that leads to renal failure): severe corneal opacities (isolated form fish eye disease, DD Tangier disease without renal involvement, and Schnyder corneal dystrophy). Familial LCAT deficiency is associated with hypertriglyceridaemia, normochromic haemolytic anaemia, and proteinuria. The prognosis for this form depends on the extent to which the disease affects renal function: the accumulation of abnormal lipoproteins in the kidneys may lead to renal insufficiency.

Acquired

- Calcium deposits in the interpalpebral cornea and conjunctiva caused by *hypercalcaemia* in renal failure. Band keratopathy can lead to vision disturbance and sometimes corneal erosion.

Treatment

In case of vision disturbing band keratopathy, one would be abrading the calcium deposits after applying chelating agents. In rare cases, corneal dystrophies will require a corneal transplant to rehabilitate the vision, as the underlying disease persists the transplant may become affected over time. In recurrent corneal erosion, applying artificial tears and bandage contact lenses is helpful. Treatment of the corneal deposits in cystinosis with cysteamine eye drops has shown promise (Gahl et al., 2000).

Iris

Congenital

- Syndromes with *aniridia*: aniridia is a rare ocular disorder. It occurs because of mutations in the *PAX6* gene on band p13 of chromosome 11. It is associated with a number of syndromes. In addition, alterations of the cornea and angle malformations can occur which lead to glaucoma, cataract formation, and retinal malformation (Lee et al., 2008).
  - *Rathbun syndrome*/hypophosphatasia: partial aniridia, unilateral renal agenesia, and mild psychomotor retardation (Sommer et al., 1974).
  - *WAGR syndrome*: the acronym stands for Wilms' tumour, aniridia, urogenital anomalies, and retardation. Wilms' tumour, or nephroblastoma, is the most frequent renal malignancy in childhood. About 1% of these children present with aniridia. The prevalence is < 1/100,000.
  - *Cat eye syndrome*, a very rare disorder, derived from a defect in chromosome 22. It includes iris coloboma and anal atresia. Choroidal or optic nerve coloboma and microphthalmia have been described. Variable renal abnormalities such as unilateral absence, unilateral or bilateral hypoplasia, and cystic dysplasia have been reported. Other systemic manifestations include pre-auricular skin tags and/or pits (which are probably the most consistent feature), congenital heart defect, and usually low-normal intelligence.
- Pupillary abnormalities:
  - *Pierson syndrome* (microcoria-congenital-nephrosis syndrome) (Zenker et al., 2004). The most characteristic ocular anomaly is microcoria. A wide range of additional abnormalities include posterior embryotoxon, megalocornea, iris hypoplasia, cataract, abnormal lens shape, posterior lenticonus, persistent fetal vasculature, retinal detachment, variable axial lengths, and glaucoma. There was high interocular and intrafamilial variability (Bredrup et al., 2008). *LAMB2* gene mutation is the underlying genetic defect for Pierson syndrome. Renal symptoms are congenital nephritic syndrome caused by diffuse mesangial sclerosis potentially leading to ESRD in the pre- or postnatal period (Hasselbacher et al., 2006).
  - *Amyloidosis*: pupillary abnormalities and oculomotor dysfunction occur.
- *Nail-patella syndrome*: a cloverleaf dark pigmentation of the central area of the iris with scalloped iris collarette (Lester sign of the iris) is a peculiar finding. Strabismus, keratoconus, microcornea, sclerocornea, microphakia, and cataracts have been described (Meyrier et al., 1990). *LMX1B* is also expressed in podocytes leading to nephritic syndrome and renal insufficiency but ESRD is less common (Bongers et al., 2005).

Acquired

- *Uveitis*:
  - TINU: a specific form of intraocular inflammation (uveitis) combined with tubulointerstitial nephritis. Ocular symptoms (redness, pain, photophobia) often precede renal disease (Mackensen and Billing, 2009).
  - Anecdotally uveitis occurs in systemic vasculitis.

Treatment

Aniridia can be treated by surgical methods which try to rebuild the iris. Other approaches include tattooing the cornea or contact lenses with an iris imprint. The visual gain depends on associated structural problems (foveal hypoplasia or nerve defects). Cataract and glaucoma secondary to aniridia generally require a surgical approach.

Anterior uveitis is treated with topical prednisolone 1% eye drops and dilating agents. Highly active anterior uveitis may require a short term of oral corticosteroids or periocular depot injections.

Lens

Congenital

- *Oculocerebrorenal syndrome of Lowe*: renal symptoms present with Fanconi syndrome. Renal manifestation can be minimal but can also reach nephritic-range proteinuria. Renal insufficiency progresses very slowly with reported ESRD in the fourth or fifth decade (Schramm et al., 2004). Congenital bilateral cataract is present at birth in all patients (Loi, 2006).
- *Alport syndrome*: anterior lenticonus (anterior protrusion of the lens): present in 20–30% affected males, develops later in the disease, but can lead to a first diagnosis of Alport syndrome when a patient presents with blurred vision. When present, anterior lenticonus is pathognomonic for Alport syndrome. May be complicated by subcapsular cataract. Usually asymptomatic retinal lesions (bilateral white or yellow granulations) will be found (Perrin et al., 1980).
- *Galactosaemia*: the changed osmotic situation in the lens caused by intracellular galactose accumulation leads to cataract in the first months of life (Schlötzer-Schrehardt and Naumann, 1997). A reversible and incomplete Fanconi syndrome can occur which disappears completely under a galactose-free diet (Geary and Schaefer, 2008).

Acquired

- Subcapsular cataracts due to steroid treatment occur in 20–30% of patients depending on dose, form of application, and length of treatment (Schlötzer-Schrehardt and Naumann, 1997).
- Cataracts due to chronic hypocalcaemia can be the presenting sign of otherwise asymptomatic renal failure (rare!!). Questionable higher frequency of cataracts in dialysis patients.

Treatment

Cataracts in older patients can be operated on with local anaesthesia easily and without special considerations. In congenital cataracts, early surgery with general anaesthesia without lens implantation and better development of the visual system has to be weighed against elevated risk of glaucoma.

Posterior segment changes

Vitreous

- *Amyloidosis*: vitreous opacities as well as retinal amyloid deposits have been confirmed histologically and may lead to secondary glaucoma (Sandgren et al., 2008). This seems to be more frequent in familiar amyloidosis than in primary, non-familiar cases (Ruprecht and Naumann, 1997).

Treatment

Vitrectomy may be required in vision disturbing opacities.

Retinal

Congenital

- *Von Hippel–Lindau disease* is an autosomal dominant neoplasia syndrome, caused by germline mutations in the *VHL* tumour suppressor gene, predisposing to ocular and central nervous system haemangioblastomas, renal cell carcinoma, and phaeochromocytoma, as well as cysts in various organs (Maher et al., 2011) (Fig. 171.2).

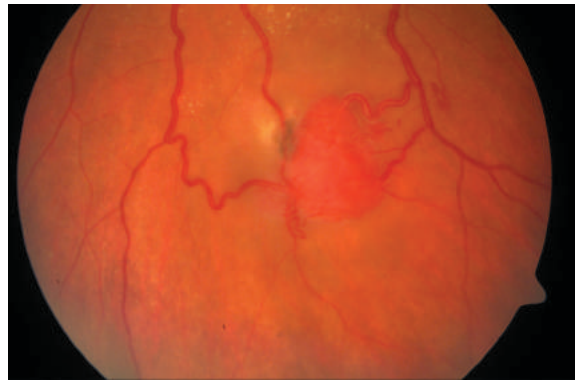

Fig. 171.2 Fundus picture of a 43-year-old female with von Hippel–Lindau disease showing retinal haemangioblastoma.

- *Oxalosis*: calcium oxalate crystals are directly injurious to renal cells and the combination of obstruction and infection related to oxalate stones lead to kidney failure and ESRD (50% at 15 years of age). Plasma oxalate concentration raises with reduced glomerular filtration rate (GFR) and calcium oxalate begins to deposit in all organ systems and serious morbidity and death can result (Hoppe and Langman, 2003). In the eye, flecked retinopathy, minute, white, round flecks of the posterior pole and near the vessels, which correspond to calcium oxalate crystals, are seen (Lasram et al., 1997).
- *Alport syndrome*: dot-and-fleck retinopathy in 85% of affected males. Always found when anterior lenticonus is present. Retinopathy is often present at the onset of renal symptoms. Macular holes have been described as a rare, later complication (Savige and Colville, 2009).
- *Retinitis pigmentosa-like disease*: tapetoretinal degeneration is characterized by a progressive deterioration of retinal receptor function (rods and cones). Typically, reduced night vision and visual field constriction are the first symptoms and are confirmed by visual field testing, electroretinography, and fundoscopy. Tapetoretinal degeneration can be found in many ciliopathies (Adams et al., 2007; and see Chapter 314). The most frequent are presented here:

  - *Nephronophthisis* (NPHP): *Senior–Loken syndrome* (SL) refers to the association of the nephronophthisis complex (the most frequent genetic cause of chronic renal failure in children) and tapetoretinal degeneration. Multiple (NPHP) genes underlie this autosomal recessive disorder, with gene products that are linked to dysfunctional cilia. NPHP is a progressive renal disease with corticomedullary cysts that almost uniformly leads to ESRD in different age groups depending on the genetically characterized. SL occurs in approximately 10–20 % of cases of NPHP. It has been described as either a childhood-onset retinitis pigmentosa or a Leber's congenital amaurosis type of abnormality and can lead to blindness in childhood (Godel et al., 1979). Several other syndromes in which NPHP and/or retinal involvement can present as a main clinical finding include Joubert, Meckel–Gruber, Cogan, and

Jeune syndromes. These syndromes show neurologic and oculomotor abnormalities.

- *Bardet–Biedl syndrome*: is characterized by rod-cone dystrophy (> 90%), truncal obesity (72%), postaxial polydactyly, cognitive impairment, male hypogonadotrophic hypogonadism, complex female genitourinary malformations, and renal abnormalities that may lead to renal failure. Cystic kidney disease and evidence of sexual infantilism with infertility are also common.
- *Alström syndrome* is a progressive autosomal recessive genetic disorder caused by specific mutations in the *ALMS1* gene affecting multiple organ systems. It may be detected at birth or in early childhood. Clinically, patients with Alström syndrome develop cone-rod dystrophy leading to eventual blindness, sensorineural deafness, and normal intelligence. Patients develop obesity, endocrine disturbances such as type 2 diabetes mellitus, dilated cardiomyopathy, and progressive renal and hepatic failure. Severe renal disease is usually a late finding; the first signs may be polyuria and polydipsia resulting from a concentrating defect secondary to interstitial fibrosis. ESRD can occur as early as the late teens.

Acquired

- *Retinopathies* in chronic kidney disease (Grunwald et al., 2010) are frequent, especially related to arterial hypertension and diabetes. AMD risk seems to be increased for patients with chronic kidney disease. In a prospective study on 1183 patients, a GFR < 60 mL/min was associated with a threefold higher risk of developing AMD than those > 60 mL/min (Liew et al., 2008).
  - Hypertensive retinopathy can manifest from mild vessel changes over signs of ischaemia (cotton-wool spots) and optic nerve swelling depending on the magnitude of blood pressure deviation.
  - A similar picture can be seen in vasculitis-associated retinopathy (systemic lupus erythematosus).
  - Diabetic retinopathy is a frequent cause of blindness and develops in many patients with diabetes correlated to disease control (HbA1c). Early signs are microaneurysms and haemorrhages and can progress to proliferative disease with severe vision impairment.
- *Serous retinal detachment* mimicking central serous chorioretinopathy in patients undergoing haemodialysis, caused by a dysfunction of the retinal pigment epithelium and electrolyte imbalance.
- Opportunistic ocular *infections* after kidney transplantation can lead to a severe, rapidly progressing, vision-threatening, retinitis, mostly caused by cytomegalovirus or other herpes viruses. PCR from ocular fluid can confirm the diagnosis and thus lead therapy.
- *Type II membranoproliferative glomerulonephritis* is characterized by the deposition of abnormal material within the glomerular basement membrane of the kidney and within Bruch's membrane in the eye. Drusen-like deposits are seen in the macular region (Appel et al., 2005). In contrast to AMD these develop at an early age (second decade). Initially, vision is not impaired, but in about 10% vision reduction occurs caused by subretinal neovascular membranes, macular detachment, or central serous retinopathy.

Treatment

Haemangioblastomas have been treated with laser coagulation, endoresection, photodynamic therapy, beam radiation, or intravitreal bevacizumab. Diabetic retinopathy is treated with laser coagulation, severe cases require vitrectomy. Hypertensive changes reverse over weeks when blood pressure is controlled. Viral retinitis can be treated systemically but intraocular drug application is preferable. Severe cases with tractive retinal detachment require surgical treatment.

Optic nerve

- *DIDMOAD* (Wolfram) syndrome (diabetes insipidus, insulin-deficient diabetes mellitus, optic atrophy and deafness), is a rare, multisystem, neurodegenerative disorder of autosomal recessive inheritance (Rigoli et al., 2011). Optic nerve atrophy with increasing circular visual field defect occurs.
- *Papillorenal syndrome* or *renal coloboma syndrome*, sometimes also called 'empty disc'. Characteristic findings consist of an absence or attenuation of the central retinal vessels within the optic nerve and the presence of multiple compensatory cilioretinal vessels. Supranasal visual field defects can be seen. The ophthalmic findings should lead to screening for renal disease (Parsa et al., 2001). The kidneys show a severe form of glomerulonephritis that may lead to renal failure (Bron et al., 1989). A mutation in the *PAX2* gene is suspected (Alur et al., 2010).

References

Abed, L., Merouani, A., Haddad, E., *et al.* (2008). Presence of autoantibodies against tubular and uveal cells in a patient with tubulointerstitial nephritis and uveitis (TINU) syndrome. *Nephrol Dial Transplant*, 23(4), 1452–5.

Adams, N. A., Awadein, A., and Toma, H. S. (2007). The retinal ciliopathies. *Ophthalmic Genet*, 28(3), 113–25.

Alur, R. P., Vijayasarathy, C., Brown, J. D., *et al.* (2010). Papillorenal syndrome-causing missense mutations in PAX2/Pax2 result in hypomorphic alleles in mouse and human. *PLoS Genet*, 6(3), e1000870.

Appel, G. B., Cook, H. T., Hageman, G., *et al.* (2005). Membranoproliferative glomerulonephritis type II (dense deposit disease): an update. *J Am Soc Nephrol*, 16(5), 1392–403.

Bongers, E. M., Huysmans, F. T., Levtchenko, E., *et al.* (2005). Genotype-phenotype studies in nail-patella syndrome show that LMX1B mutation location is involved in the risk of developing nephropathy. *Eur J Hum Genet*, 13(8), 935–46.

Bredrup, C., Matejas, V., Barrow, M., *et al.* (2008). Ophthalmological aspects of Pierson syndrome. *Am J Ophthalmol*, 146(4), 602–11.

Bron, A. J., Burgess, S. E., Awdry, P. N., *et al.* (1989). Papillo-renal syndrome. An inherited association of optic disc dysplasia and renal disease. Report and review of the literature. *Ophthal Paediatr Genet*, 10(3), 185–98.

Brownstein, M. H., Elliott, R., and Helwig, E. B. (1970). Ophthalmologic aspects of amyloidosis. *Am J Ophthalmol*, 69(3), 423–30.

Colville, D. J. and Savige, J. (1997). Alport syndrome. A review of the ocular manifestations. *Ophthalmic Genet*, 18(4), 161–73.

Edwards, A. O., Ritter, R., 3rd, Abel, K. J., *et al.* (2005). Complement factor H polymorphism and age-related macular degeneration. *Science*, 308(5720), 421–4.

Gahl, W. A., Kuehl, E. M., Iwata, F., *et al.* (2000). Corneal crystals in nephropathic cystinosis: natural history and treatment with cysteamine eyedrops. *Mol Genet Metab*, 71(1–2), 100–20.

Geary, D. F. and Schaefer, F. (eds.) (2008). *Comprehensive Pediatric Nephrology*. Philadelphia, PA: Mosby.

Godel, V., Iaina, A., Nemet, P., *et al*. (1979). Retinal manifestations in familial juvenile nephronophthisis. *Clin Genet*, 16(4), 277–81.

Grunwald, J. E., Alexander, J., Maguire, M., *et al*. (2010). Prevalence of ocular fundus pathology in patients with chronic kidney disease. *Clin J Am Soc Nephrol*, 5(5), 867–73.

Guthoff, R. F., Zhivov, A., and Stachs, O. (2009). In vivo confocal microscopy, an inner vision of the cornea—a major review. *Clin Experiment Ophthalmol*, 37(1), 100–17.

Hasselbacher, K., Wiggins, R. C., Matejas, V., *et al.* (2006). Recessive missense mutations in LAMB2 expand the clinical spectrum of LAMB2-associated disorders. *Kidney Int*, 70(6), 1008–12.

Hoppe, B. and Langman, C. B. (2003). A United States survey on diagnosis, treatment, and outcome of primary hyperoxaluria. *Pediatr Nephrol*, 18(10), 986–91.

Jais, J. P., Knebelmann, B., Giatras, I., *et al*. (2003). X-linked Alport syndrome: natural history and genotype-phenotype correlations in girls and women belonging to 195 families: a "European Community Alport Syndrome Concerted Action" study. *J Am Soc Nephrol*, 14(10), 2603–10.

Klein, R. J., Zeiss, C., Chew, E. Y., *et al*. (2005). Complement factor H polymorphism in age-related macular degeneration. *Science*, 308(5720), 385–9.

Knopp, E. A. and Cowper, S. E. (2008). Nephrogenic systemic fibrosis: early recognition and treatment. *Semin Dial*, 21(2), 123–8.

Lasram, L., Kammoun, A., Karray, T., *et al.* (1997). [Ocular signs of primary hyperoxaluria type I]. *J Fr Ophtalmol*, 20(4), 258–62.

Lee, H., Khan, R., and O'Keefe, M. (2008). Aniridia: current pathology and management. *Acta Ophthalmol*, 86(7), 708–15.

Levinson, R. D., Park, M. S., Rikkers, S. M., *et al*. (2003). Strong associations between specific HLA-DQ and HLA-DR alleles and the tubulointerstitial nephritis and uveitis syndrome. *Invest Ophthalmol Vis Sci*, 44(2), 653–7.

Liew, G., Mitchell, P., Wong, T. Y., *et al*. (2008). CKD increases the risk of age-related macular degeneration. *J Am Soc Nephrol*, 19(4), 806–11.

Liu, J., Colville, D., Wang, Y. Y., *et al*. (2009). The dot-and-fleck retinopathy of X linked Alport syndrome is independent of complement factor H (CFH) gene polymorphisms. *Br J Ophthalmol*, 93(3), 379–82.

Loi, M. (2006). Lowe syndrome. *Orphanet J Rare Dis*, 1, 16.

Mackensen, F. and Billing, H. (2009). Tubulointerstitial nephritis and uveitis syndrome. *Curr Opin Ophthalmol*, 20(6), 525–31.

Mackensen, F., David, F., Schwenger, V., *et al*. (2011). HLA-DRB1*0102 is associated with TINU syndrome and bilateral, sudden-onset anterior uveitis but not with interstitial nephritis alone. *Br J Ophthalmol*, 95(7), 971–5.

Maher, E. R., Neumann, H. P. and Richard, S. (2011). von Hippel-Lindau disease: a clinical and scientific review. *Eur J Hum Genet*, 19(6), 617–23.

Meyrier, A., Rizzo, R., and Gubler, M. C. (1990). The nail-patella syndrome. A review. *J Nephrol*, 2, 133–40.

Parsa, C. F., Silva, E. D., Sundin, O. H., *et al.* (2001). Redefining papillorenal syndrome: an underdiagnosed cause of ocular and renal morbidity. *Ophthalmol*, 108(4), 738–49.

Perrin, D., Jungers, P., Grünfeld, J. P., *et al*. (1980). Perimacular changes in Alport's syndrome. *Clin Nephrol*, 13(4), 163–7.

Rhys, C., Snyers, B., and Pirson, Y. (1997). Recurrent corneal erosion associated with Alport's syndrome. Rapid communication. *Kidney Int*, 52(1), 208–11.

Rigoli, L., Lombardo, F., and Di Bella, C. (2011). Wolfram syndrome and WFS1 gene. *Clin Genet*, 79(2), 103–17.

Ruprecht, K. W. and Naumann, G. O. H. (1997). Auge und Allgemeinkrankheiten. In G. O. H. Naumann (ed.) *Pathologie des Auges* (Vol. 2), pp. 1452–546. Heidelberg: Springer.

Sandgren, O., Kjellgren, D., and Suhr, O. B. (2008). Ocular manifestations in liver transplant recipients with familial amyloid polyneuropathy. *Acta Ophthalmol*, 86(5), 520–4.

Savige, J. and Colville, D. (2009). Opinion: Ocular features aid the diagnosis of Alport syndrome. *Nat Rev Nephrol*, 5(6), 356–60.

Schlötzer-Schrehardt, U. and Naumann, G. O. H. (1997). Linse. In G. O. H. Naumann (ed.) *Pathologie des Auges* (Vol. 2), pp. 845–954. Heidelberg: Springer.

Schramm, L., Gal, A., Zimmermann, J., *et al.* (2004). Advanced renal insufficiency in a 34-year-old man with Lowe syndrome. *Am J Kidney Dis*, 43(3), 538–43.

Smith, J. R., Mackensen, F., and Rosenbaum, J. T. (2007). Therapy insight: scleritis and its relationship to systemic autoimmune disease. *Nat Clin Pract Rheumatol*, 3(4), 219–26.

Sodi, A., A. Ioannidis, A., and Pitz, S. (2006). Ophthalmological manifestations of Fabry disease. In A. Mehta, M. Beck, and G. Sunder-Plassmann (eds.) *Fabry Disease: Perspectives from 5 Years of FOS*, pp. 249–61. Oxford: Oxford PharmaGenesis.

Sommer, A., Rathbun, M. A., and Battles, M. L. (1974). Letter: A syndrome of partial aniridia, unilateral renal agenesis, and mild psychomotor retardation in siblings. *J Pediatr*, 85(6), 870–2.

Wakaki, H., Sakamoto, H., and Awazu, M. (2001). Tubulointerstitial nephritis and uveitis syndrome with autoantibody directed to renal tubular cells. *Pediatrics*, 107(6), 1443–6.

Zenker, M., Tralau, T., Lennert, T., *et al.* (2004). Congenital nephrosis, mesangial sclerosis, and distinct eye abnormalities with microcoria: an autosomal recessive syndrome. *Am J Med Genet A*, 130A(2), 138–45.

CHAPTER 172

The patient with renal cell cancer

Tim Eisen

Introduction

At the end of the twentieth century, the medical management of renal cancer was felt to be a Cinderella subject. Treatments were predominantly based on immunotherapy which benefited a small proportion of patients at the expense of considerable toxicity for all, including the large majority of patients who had no benefit from immunotherapy. A very small but tantalizing proportion of patients were cured by immunotherapy. Since we had no means of reliably selecting people in this small minority who benefit greatly from immunotherapy, the field was felt to be a generally rather hopeless one. There has been a remarkable change over the last decade in that six new therapies have been approved following successful clinical trials. The majority of patients have some degree of tumour control and interestingly, it appears that many of the paradigms for treatment of solid tumours do not apply to renal cell cancer in the modern era. For example, failure of a particular treatment modality for a solid malignancy would normally prompt a switch to another modality and no attempt to gain further benefit for the patient from the failed therapy. For reasons which are not yet clear, it appears possible to use the same modality of treatment repeatedly in renal cancer with a significant chance of further benefit for the patient. Anti-angiogenic treatment is also remarkable for another reason. There can be few areas of medicine where predictions have proved so wrong. The received wisdom was that anti-angiogenic treatments would be very well tolerated; that they would be easy to combine with other treatments; that they would need to be used continuously; and that they would result in tumour stabilization but not shrinkage. None of these predictions have stood the test of time. Renal cancer is the best example of successful anti-angiogenic treatment in oncology but treatment is not curative. The mainstay of curative treatment remains surgery.

Surgical advances have been most impressive with significantly better patient outcomes and reduced length of stay from open and laparoscopic procedures. The key role of surgery in the treatment of limited disease is unchallenged. However, the role of surgery in patients with metastatic disease which was established for fit patients in the immunotherapy era is no longer clear. There are no convincing data suggesting that patients with an asymptomatic primary currently benefit from surgery as part of a therapeutic programme involving standard tyrosine kinase inhibitor (TKI) treatment.

Renal cancer is now one of the most active areas of oncology as we seek to lift our game and provide greater benefit for patients. This chapter will summarize the key points in the management of renal cell cancer and outline the current key research questions.

Aetiology

Most renal cancers are sporadic and do not result from inherited mutations. Inactivation of the von Hippel–Lindau (VHL) gene by deletion, mutation, or silencing of the gene by methylation, occurs in the large majority of clear cell carcinomas (Gnarra et al., 1994). More recently, mutations in chromatin remodelling genes, most notably *PBRM1*, have been identified (Varela et al., 2011).

Tobacco smoking is the best-established risk factor for renal cancer, with around a quarter of disease appearing to be attributable to smoking.

Other environmental risk factors are less obviously linked to renal cancer although some studies have implicated dietary factors, obesity, hypertension, and diabetes mellitus.

Epidemiology

Both the incidence and mortality of renal cancer have been increasing for several decades across the world, although there have been recent encouraging signs that rates have stabilized (Chow et al., 2010). The reasons for this increase are only partially understood. It is clear that with up to 70% of diagnoses in Western Europe being made incidentally during investigations for other medical problems, that there may be a stage shift accounting for part of the apparent rise in incidence. However, this would not account for the increased mortality from disease and there does appear to be a genuine increase both in incidence and mortality from renal cancer. The reasons for this increase are not understood. Although renal cancer is commonest between the ages of 50 and 75, it is well recognized in younger patients. Approximately twice as many men are affected as women. The incidence of renal cancer does vary around the globe, being highest in North America and Europe. Rates may be slightly lower in Southern Europe and Australia and are lowest in Asia, Africa, and Central and Southern America. Renal cancer accounts for approximately 3% of the total cancer burden with an incidence of around 8 per 100,000 people. In a country the size of the United Kingdom, this results in around 10,000 new cases per annum.

Clinical features

With the increasing use of non-invasive imaging, the proportion of cases diagnosed incidentally has increased from approximately 10% in 1970 to around 60% at the turn of the century. It is likely that this trend is continuing (Sunela et al., 2010). Thus there may be no presenting clinical features at diagnosis. Certainly the classical triad of haematuria, loin pain, and a palpable flank mass is now rare.

Table 172.1 The Bosniak classification of renal cysts

Category	Features	Risk of malignancy and management
I	Benign simple cyst Thin wall, no enhancement, no septa, no solid component	Benign No further follow-up
II	Benign cystic lesion Thin wall, thin septa, minimal enhancement	Benign No further follow-up
IIF	Complicated cystic lesions Minimal thickening of wall or septa, no enhancing soft tissue. Also hyperdense non-enhancing cyst > 3 cm	5% risk of malignancy Requires regular imaging follow-up
III	Indeterminate mass Thick, enhancing wall or septa	50% risk of malignancy Resect
IV	Malignant cystic mass Thick, enhancing wall or septa with enhancing soft tissue component	Malignant Resect

Any of these three symptoms may suggest renal cancer. In addition, other presenting features may include local effects, such as obstruction to the venous system, caused by the renal cancer growing along the renal vein and into the inferior vena cava and then cranially.

Distant effects are common with renal cell cancer, with approximately a quarter of patients presenting with metastatic disease, especially in lymph node, lung, and bone. In addition, renal cancer is frequently a highly metabolically active tumour with a wide range of paraneoplastic phenomena described. Secretion of vasoactive substances by the tumour or other effects of the tumour on normal renal tissue may lead to hypertension whilst other hormonal effects include polycythaemia due to increased erythropoietin production and hypercalcaemia due to parathyroid hormone-related peptide.

Systemic features most commonly include weight loss, fever, night sweats, and fatigue and are generally held to be adverse features. Rarely, hepatic dysfunction not related to metastatic involvement is seen. These paraneoplastic syndromes may all resolve following nephrectomy in cases of limited disease. The detection and characterization of renal masses is a specialist area which has assumed even greater importance given the significant rise in the incidental diagnosis of renal abnormalities. The radiological assessment of these lesions is discussed in greater detail in the next section.

Cystic renal lesions are very common and it is necessary to distinguish between benign and malignant cysts. The Bosniak classification of renal cysts is most commonly used to categorize lesions with associated risk of malignancy (Table 172.1) (Bosniak, 1986).

It is important to note that even malignant lesions can be very effectively treated by surgery and that a tumour with a diameter of < 3 cm very rarely results in metastatic disease. Treatment is guided by a number of risk scoring systems appropriate to different stages of disease. I shall not consider a patient's operability in this chapter although clearly that is a most important consideration in determining the optimal treatment strategy. The Leibovich or Mayo scoring system has become increasingly widely used with the advent of adjuvant therapy trials. The risk of relapse is modelled by considering tumour T-stage, presence of necrosis, involvement of lymph nodes, and absolute size of the tumour in excess of 10 cm. This allows allocation of patients to different risk groups commonly simplified to low, intermediate, or high risk (Table 172.2) (Fig. 172.1).

Table 172.2 Stage, size, grade, and necrosis (SSIGN or Leibovich) scoring system for metastasis-free survival following nephrectomy for renal cell cancer

Characteristic	Result and score
T-stage	pT1a = 0; pT1b = 2; pT2 = 3; pT3a–4 = 4
Regional lymph nodes	pNx or pN0 = 0; pN1 or pN2 = 2
Tumour size	< 10 cm = 0; > 10 cm = 1
Nuclear grade	1 or 2 = 0; 3 = 1; 4 = 2
Histological tumour necrosis	Absent = 0; present = 1
Maximum total	10

The most commonly used risk scoring system for advanced disease is the Memorial Sloan Kettering Cancer Center (MSKCC) score which was originally developed in the immunotherapy era. The MSKCC system assigns patients to good, intermediate, or poor risk according to the number of adverse prognostic features present: Karnofsky performance status < 80%, lactate dehydrogenase > 1.5 times the upper limit of normal, haemoglobin below lower limit of normal, corrected calcium above upper limit of normal, and the absence of a prior nephrectomy/time from diagnosis to treatment for metastatic disease of < 1 year. Patients with no risk factors had a median survival of 20 months, whilst those with one to two risk factors had a median survival of 10 months, and those with three or more risk factors had a survival of just 4 months (Motzer et al., 1999). The MSKCC scoring system was devised during the era of immunotherapy and has been revised recently by clinicians at the Cleveland Clinic to reflect the TKI era (Heng et al., 2009) Karnofsky performance status < 80%, haemoglobin below lower limit of normal, corrected calcium above upper limit of normal, and time from diagnosis to treatment of < 1 year were all validated and were joined by neutrophil count greater than the upper limit of normal and platelets greater than the upper limit of normal as risk factors. Those with no risk factors had a 2-year survival of 75% (median survival was not reached at the time of diagnosis), whilst those with one or two risk factors had a median survival of 27 months, and those with three to six risk factors had a median survival of 9 months.

The Kidney Cancer Association has recently published an updated scoring model which can be further developed in an iterative way once new data become available. The scoring system is available online (<http://www.kidneycancer.org/rcc-risk-calc>).

Investigations

The purpose of investigations for renal cancer is (1) to establish a diagnosis, (2) to assess the extent of disease and hence treatment intent and possible modalities of treatment, (3) to assess the patient's suitability for these treatment options, and (4) to monitor the effectiveness of treatment and the patient's ability to tolerate treatment.

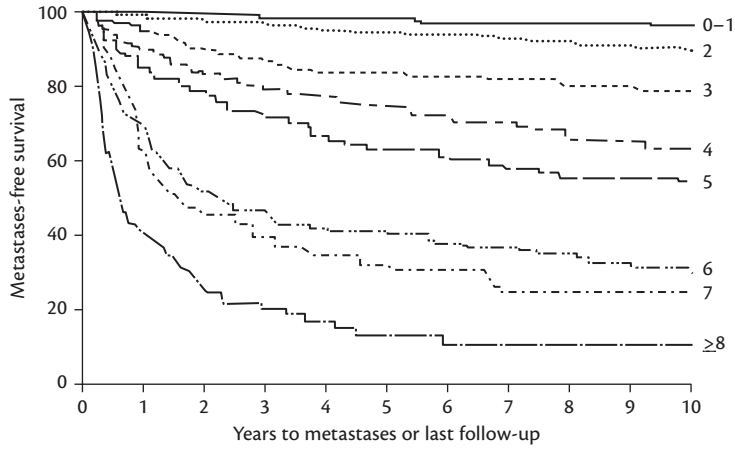

Fig. 172.1 Actuarial metastasis-free survival after resection of renal cancer stratified by scoring system.
Reproduced from Leibovich, B. C., Blute, M. L., Cheville, J. C., *et al.* (2003). "Prediction of progression after radical nephrectomy for patients with clear cell renal cell carcinoma". *Cancer*, 97, 1663–71.

Assessment of fitness and co-morbidities

Clinical assessment of the patient with renal cancer must establish a patient's general fitness or performance status, the presence of symptoms or signs suggestive of distant metastatic disease, and the presence of other medical problems which could complicate management. Relevant co-morbidities such as hypertension, pre-existing cardiac disease, and diabetes may predispose patients to cardiovascular toxicity of agents such as the TKIs. If any of these factors are present, then echocardiography should be considered to establish whether cardiac function is sufficient to make TKI treatment a reasonably safe proposition. There are few reliable data relating to this although a generally accepted safety parameter is that the left ventricular ejection fraction should exceed 50% (Schmidinger et al., 2008). The presence of renal insufficiency is an important factor in determining whether surgery is possible, or even desirable if the patient is likely to become dialysis dependent as a consequence. Accurate assessment of renal function and the relative contribution of the area to be resected are possible by calculating split renal function by dimercaptosuccinic acid (DMSA) scan.

Radiological assessment of the primary tumour

Radiological assessment of the tumour itself is most commonly performed by computed tomography (CT). Where possible, CT scans are taken before and after contrast enhancement to provide information on calcification and the presence of fat within a lesion, as well as providing information on vascular supply. Ultrasound may be particularly helpful in distinguishing between solid and cystic renal lesions.

Magnetic resonance imaging (MRI) is less commonly used in the assessment of renal lesions, although it is preferred in individuals who need repeated examinations, such as those with VHL disease. MR angiography may also provide extremely useful information in imaging tumour and normal kidney vasculature prior to renal surgery. MRI may occasionally be necessary to assess tumour thrombus in the inferior vena cava. In addition, transoesophageal ultrasound may be necessary to assess tumour if it extends cranially towards the right atrium.

Nuclear medical techniques such as positron emission tomography (PET) and bone scintigraphy are unreliable in renal cancer. Renal cancers may be slow growing and not detected on PET scan whilst the bone lesions are often purely lytic and do not reliably result in tracer uptake.

Staging

Staging of the primary tumour, regional lymph nodes, and presence or absence of distant metastases is vital, irrespective of which treatment modality is employed. The most commonly used staging system now is the TNM (tumour, node, metastasis) staging classification (Edge et al., 2010).

Spread to lymph nodes may be detected on CT or MRI and reliably so in nodes > 2 cm. More distant metastases due to haematogenous spread are usually multifocal but oligometastatic disease is a well-recognized phenomenon in renal cell cancer, and requires specific management as described in the 'Treatment and outcome' section. Pulmonary metastases are the most common site of distant disease in renal cancer. They are classically described as cannonball metastases. Thoracic metastases from renal cancer are often found in the mediastinal lymph nodes, pleura, and endobronchially.

Radiological assessment of rate of progress and response to treatment

Imaging is central to the follow-up of patients after nephrectomy or ablation of a renal lesion and to the assessment of response to systemic treatment. The main purpose of CT imaging following a procedure with curative intent is to determine whether there is any evidence of local or distant relapse. Following nephrectomy, the most likely site of local relapse is in the renal bed and associated lymph nodes. This is most likely to occur with 3 years of nephrectomy and further resection may still be considered with curative intent. In the case of follow-up after ablation, the radiologist is searching for evidence of enhancing soft tissue consistent with viable tumour. Further ablation may then be considered.

The assessment of response to medical treatment for renal cancer differs significantly from the assessment for other malignant solid tumours. In renal cancer, the treatments are often able to de-vascularize lesions and prevent growth but may not cause significant tumour volume reduction. For this reason, the standard response evaluation criteria in solid tumours (RECIST) need to be

used with caution when determining the success of treatment or otherwise. Nevertheless, the RECIST criteria for response are frequently quoted in papers relating to novel therapies in renal cancer and are worth noting for this reason alone (Eisenhauer et al., 2009). Complete response indicates the complete resolution of all target lesions, partial response means at least 30% reduction in the sum of measured tumour diameters. Progressive disease means an increase of at least 20% in the sum of tumour diameters or the appearance of new lesions and stable disease is the case were none of the above apply.

Progression-free survival currently remains the best single marker of long-term patient outcome. This subtle, though important difference from the assessment of other solid malignancies reflects the continuous maintenance role of systemic therapies as opposed to the intermittent tumour shrinking role of chemotherapies used in other solid malignancies, such as breast and lung cancer.

Liver metastases from renal cancer may produce hypervascular or isodense lesions which in response to anti-angiogenic treatment may become hypodense and more easily seen. This has become a particular problem where successful anti-angiogenic treatment apparently results in more disease in the liver, whereas in fact pre-existing disease has merely become easier to see.

Biopsy

The histological diagnosis is often not available until after a surgical resection of primary disease. However, where nephrectomy is not intended, or where there is reason to think that renal cell carcinoma is not the correct diagnosis, then a biopsy should be performed.

Differential diagnosis

Differential diagnosis of renal cancer is mainly determined by radiological appearances on presentation. It is important to note that a nephrectomy is often performed without histological diagnosis prior to surgery where the appearances are highly suggestive of a renal cell carcinoma. This is true for localized disease but may also be the case where there is low-volume metastatic disease in an otherwise fit patient. Awareness of other diagnoses requiring very different management is therefore important. The key differential diagnoses are transitional cell carcinoma, usually found in the pelvis and collecting systems, lymphoma, often associated with very bulky lymph node involvement, Wilms tumour, usually found in young children, oncocytoma with a characteristic cartwheel appearance on CT, and angio-myolipomas which usually, but not always, contain macroscopic fat.

Aetiology and pathogenesis

Most renal cancers are sporadic and do not result from inherited mutations. Nevertheless, renal cancer provides an example of where understanding of the common sporadic disease derives from an understanding of the molecular pathogenesis of inherited variants. Patients who inherit a mutation in the *VHL* gene develop VHL disease, which includes a 70% lifetime risk of clear cell renal cancer. The tumours contain a somatic mutation in the other copy of the *VHL* gene, and are often bilateral and multifocal. Inactivation of both copies of the *VHL* gene also occurs in the large majority of sporadic clear cell carcinomas (Gnarra et al., 1994). The key target of the *VHL* gene product is hypoxia-inducible factor alpha (HIF) which regulates angiogenesis, proliferation, and other important cellular functions. When the *VHL* gene product is not functioning, HIF is hyperactive and leads to deregulated angiogenesis (Cockman et al., 2000). More recently, mutations in chromatin remodelling genes, most notably *PBRM1*, have been identified (Varela et al., 2011). Mutations in *VHL* and chromatin-remodelling genes are not exclusive. The interaction between them is not clear although one theory is that *VHL* mutations alone would tend to lead to cellular senescence, a process abrogated by abnormalities in the chromatin-remodelling machinery of the cell. Thus if both *VHL* and chromatin-remodelling mutations are present, the tumour cell is able to induce neo-angiogenesis and proliferate without becoming senescent.

Tobacco smoking is the best-established risk factor for renal cancer (McLaughlin et al., 1984). There have been several studies conducted and the attributable risks vary in these studies with around a quarter of disease appearing to be attributable to smoking. Some studies have shown that the risk of renal cancer is proportional to the amount of smoking and that this risk reduces with smoking cessation.

Other environmental risk factors are less obviously linked to renal cancer although some studies have implicated dietary factors, obesity, hypertension, and diabetes mellitus (Benichou et al., 1998). There is no clarity at present about relationships between drugs for hypertension or pain control and risk of renal cancer, with contradictory findings in multiple studies.

It is now evident that there are several distinct subtypes of renal cancer, each associated with specific molecular abnormalities. Although several classification systems have been used for renal cancer, the most commonly used is the Heidelberg classification.

Tumours are divided into benign and malignant tumours. Clear cell carcinoma is the commonest type of malignant tumour and accounts for approximately three-quarters of the disease. A clear cell appearance is a pathological descriptive term. Typically, clear cell cancers are heavily laden with lipid and glycogen which is removed during the tissue processing procedure, giving an artefactual 'clear cell' appearance. For this reason, some pathologists may prefer to use the term conventional renal cancer. Papillary renal cancer accounts for approximately 15% of the disease. The patient with papillary renal cell carcinoma may show multifocal abnormalities in the kidney associated with multiple papillary renal adenomas. There are two distinct subtypes of papillary renal cell carcinoma. Type 1 is commonest and appears to have a better prognosis than type 2, which may have an extremely aggressive course. Chromophobe renal cell carcinomas account for approximately 5% of disease and are usually more indolent tumours than the commoner variants. Collecting duct carcinomas, also known as Bellini tumours, are related to the aggressive medullary carcinoma associated with sickle-cell trait. These are rare tumours and account for approximately 1% of the disease burden.

The molecular classification of renal cancer is described in detail in Chapter 328. In this chapter I shall provide only a brief background to the pathogenesis, concentrating on aspects which explain the activity of novel targeted therapies in this disease. Whilst only around 2% of renal cancers occur in people who have an inherited predisposition, the same genetic alterations underlie the much commoner sporadic renal cancer. The individual genes most implicated in each tumour type are illustrated in Table 172.3. VHL syndrome results from mutations in the *VHL* gene. Depending on the exact type of mutation, patients may be allocated to one of several

Table 172.3 Heidelberg classification of renal tumours

Lesion	Characteristics	Gene implicated	Locus
Benign tumours			
Papillary renal adenoma	Common. Similar to low-grade papillary RCC		
Metanephric adenoma	Rare. Erythropoietin expression		
Oncocytoma	Cartwheel appearance on CT	BHD	17p11
Malignant tumours			
Clear cell carcinoma	75% of cases	VHL *SWI/SNF*	3p25
Papillary carcinoma	15% of cases	Type 1 *c-MET* Type 2 *FH*	7q31 1q42
Chromophobe carcinoma	5% of cases	*BHD*	17p11
Collecting duct carcinoma	Aggressive and rare		
Unclassified			

subtypes of VHL syndrome according to their risk of developing renal cancer and associated lesions such as haemangioblastomas of the eye and central nervous system, phaeochromocytomas, and pancreatic islet cell tumours. The most important function of the *VHL* gene product is its central role in the regulation of the transcription factor HIF. Deregulation of this factor results in uncontrolled expression of hypoxia target genes such as vascular endothelial growth factor, platelet-derived growth factor, and erythropoietin.

The *c-MET* gene encodes the cell surface receptor for the hepatocyte growth factor which is assuming greater importance in the management of a number of solid malignancies. It is not yet known whether targeting of this receptor will be an effective therapeutic intervention. Type 2 papillary renal cell cancer is associated with inactivating mutations in the fumarate hydratase gene which is also thought to be important in the sporadic variety of this disease. The inherited form is often associated with early uterine fibroids and cutaneous leiomyomatosis. The renal lesion in hereditary leiomyomatosis can be extraordinarily aggressive.

The Birt–Hogg–Dubé (BHD) syndrome of multiple lung cysts is associated with the development of renal tumours including chromophobe, oncocytoma, mixed chromophobe and oncocytoma, and clear cell. The BHD locus encodes the protein folliculin which is currently being intensively studied to elucidate its function.

Treatment and outcome

There are four fundamental questions which a clinician treating a patient with renal cell carcinoma will consider. First, what is the nature and extent of the disease? Second, what is the primary objective of treatment? Is it to cure the patient, to prolong their life, or to palliate their symptoms? Third, what options theoretically exist for this patient to achieve this objective? Fourth, which of these treatment options is most appropriate for the particular patient in front of you? The patient's wishes, performance status, and co-morbidities will all need to be taken into account in answering the fourth question.

The general outcome of treatment is considered in the section on clinical features in this chapter. In this section, I will focus on answering the third question concerning the available options and the efficacy and toxicity data supporting these options.

Curative intent

If the patient is operable and has resectable disease, then the intent of treatment would be to cure the patient and the treatment of choice would be partial nephrectomy or nephron-sparing nephrectomy if possible, or open or laparoscopic radical nephrectomy if the lesion could not be resected by a partial nephrectomy. Key considerations in determining whether a lesion can be resected by partial nephrectomy include tumours < 7 cm in diameter and preferably lesions which are at either one of the poles of the kidney and do not involve the renal pelvis (Gill et al., 2007). Although partial nephrectomy can be performed laparoscopically, this is largely the preserve of extremely specialized surgeons. A laparoscopic procedure may result in a longer ischaemic time than an open procedure, resulting in greater loss of nephron function and negating the rationale for partial nephrectomy (Patard et al., 2007). Where there is limited disease but the patient is not operable or refuses surgery, then ablation should be considered for tumours up to 4 cm in size. Techniques include radiofrequency ablation and cryotherapy, in which probes are inserted percutaneously to access the renal cancer and the renal cancer tissue with a thin margin of normal tissue surrounding it, is destroyed *in situ*. Follow-up after ablation needs to be rigorous and is aimed at detecting evidence of active disease recurring locally (Breen et al., 2007). Ablation and nephrectomy have never been directly compared in a clinical trial although studies are ongoing. Where there appears to be a high surgical risk of intraoperative bleeding, embolization may be performed prior to nephrectomy to mitigate this risk.

At the time of writing (2015) there is no indication for any adjuvant treatment following resection of a renal cancer. Large studies are being performed investigating the value of adjuvant treatment with TKIs or mammalian target of rapamycin (mTOR) inhibitors.

Prolonging survival

Renal cancer has a very variable natural history and many patients will remain well without any active treatment for long periods, whilst others will progress quickly and require aggressive intervention. Factors which point to an aggressive tumour include systemic symptoms of weight loss, night sweats, and hypercalcaemia. In the absence of these symptoms, it may be very reasonable to observe the patient for a period of time with a repeat scan usually after 3 months to assess the rate of change. There are no data suggesting that early systemic management of indolent, asymptomatic disease is of greater benefit than observing the patient and starting treatment once significant or symptomatic progression is encountered. For many patients who are frail or have co-morbidities, observation with the intention of palliating symptoms as they occur may be the best strategy.

Surgery does have a role in selected patients even in the metastatic setting. It is well recognized that some renal carcinomas behave as oligometastatic disease. That is, disease which progresses at just one or two sites. Where this pattern is established, a surgical

Table 172.4 Treatment options in metastatic renal cancer

	Good risk	Intermediate risk	Poor risk
First line	Sunitinib Pazopanib IL-2	Sunitinib Pazopanib	Temsirolimus
Second line	Sorafenib Everolimus Axitinib	Sorafenib Everolimus Axitinib	Few data

approach may result in medium- to long-term control of disease (Kavolius et al., 1998). Factors which select patients for a good outcome include a disease-free interval > 12 months, a solitary site of disease, and age < 60 years. Before embarking on such a strategy, it is necessary to establish that the renal cancer is indeed behaving in this oligometastatic way. A typical history would be of a patient who has a nephrectomy then a disease-free interval in excess of a year, when a routine scan identifies a solitary metastasis in the lung. Intervention immediately on diagnosis of an apparently solitary metastasis may result in a significant number of patients having a pointless procedure where further metastases develop in the next few months following surgery. A short period of observation of 3–6 months followed by metastasectomy may be of significant benefit in these selected patients. Sites of disease which lend themselves to this approach are lung, lymph node, and bone disease. One other site where surgery for oligometastatic disease may be performed but often without an observation period, is brain disease, where other means of control of metastasis are relatively ineffective.

Systemic treatment options have been investigated in a number of first-, second-, and third-line settings. The treatment grid in Table 172.4 shows where the phase 3 data have supported various treatment options.

There are currently no ways of selecting individual TKIs or mTOR inhibitors for individual patients although attempts better to target these treatments continue. Sunitinib (Motzer et al., 2007), sorafenib (Escudier et al., 2007), pazopanib (Sternberg et al., 2010), axitinib (Rini et al., 2011), and bevacizumab plus interferon (Rini et al., 2004; Escudier et al., 2007b) have all been found significantly to increase progression-free survival in the various settings in which they have been investigated. All of these agents have an approximately 75% chance of causing tumour shrinkage or reduction. Sunitinib, pazopanib, axitinib, and bevacizumab plus interferon have an approximately 35% chance of causing a partial response by RECIST and each results in 10–14 months of progression-free survival in the first-line setting. At the time of writing (2015), sunitinib and pazopanib are considered the standard first-line agents for metastatic renal cell carcinoma. The combination of the vascular endothelial growth factor antibody bevacizumab with interferon similarly doubles progression-free survival compared to interferon alone. Interestingly, in combination with bevacizumab, low dose interferon appears to be as effective as high-dose interferon and better tolerated. The mTOR inhibitors temsirolimus (Hudes et al., 2007) and everolimus (Motzer et al., 2009) have provided progression-free survival benefits and an overall survival benefit in the case of temsirolimus in the settings in which they have been investigated. It is worth noting that the placement of these agents depends very largely on the entry criteria of the studies rather than any particular scientific rationale limiting them to that particular group of patients.

The toxicities of TKIs and mTOR inhibitors needs to be considered carefully as these are used in the maintenance setting. The main toxicities of TKIs are hypertension, skin rash, fatigue, stomatitis, and a variety of less common toxicities. The key features of toxicity management are early intervention and employment of prophylactic measures. Whilst all of the TKIs can lead to these side effects, individual TKIs have a greater or lesser propensity to each. Thus sorafenib tends to cause the worst skin reaction, sunitinib tends to cause the worst fatigue and stomatitis, axitinib causes the greatest degree of hypertension, and pazopanib the greatest degree of liver dysfunction. The mTOR inhibitors have overlapping toxicities but also can cause hypercholesterolaemia and hypertriglyceridaemia with hyperglycaemia. In general terms, problems arising from use of these maintenance agents are caused by too much treatment rather than too little. It is notable that early interruption of treatment will often result after a couple of days in a marked improvement in the patient's condition so that treatment can be resumed without difficulty. However, if the severity of the side effect is allowed to worsen, then patients may need to be off treatment for prolonged periods or even permanently.

The role of immunotherapy has reduced very markedly over the past 5 years. Many centres do not offer immunotherapy at all whilst others continue to use it for selected groups. The reason that immunotherapy remains an option at all is that it is well recognized that a small group of patients do extremely well on immunotherapy with maintained complete responses equating to cure. The percentage of patients in this group is only around 1–2% of the whole renal cancer group and therefore the high toxicity of treatment with the option of better tolerated treatment which is more likely to work in the large majority of patients has reduced the uptake of immunotherapy significantly. A very limited number of centres offer high-dose interleukin-2 therapy which may cure up to 20% of a highly selected group of patients but which has not been proven in a prospective randomized phase 3 trial (Klapper et al., 2008).

There is extensive research in the use of novel immunotherapies such as autologous tumour vaccines and T-cell checkpoint inhibitors such as antibodies to PD-1, PD-L1, and CTLA4 which relieve the control of autoimmunity and may induce host-versus-tumour immune reactions. These approaches remain experimental at the time of writing.

Palliative measures and special circumstances

It is important to realize that debulking by surgery or destruction of disease by systemic therapy may provide major palliative benefit. For example, a patient with metastatic disease who has a painful and bleeding primary lesion may benefit from a palliative nephrectomy. Embolization may be performed to palliate symptoms for patients with advanced symptomatic renal cell carcinoma who are not operable.

Equally, high-quality palliation of symptoms will form an important part of the management of nearly all patients with disseminated disease.

There are data suggesting that the use of a bisphosphonate such as zoledronate will slow down the development of new bony metastases once a patient has developed hypercalcaemia or a known bone metastasis. As well as this, zoledronate can be of palliative benefit in reducing pain and the symptoms of hypercalcaemia. The regular

use of zoledronate for patients who have experienced symptoms of this sort has become the standard of care.

The safety of TKIs in patients with brain metastases has only been established following whole-brain radiotherapy. It is therefore generally accepted that prior to using TKIs in patients with central nervous system disease, whole-brain radiotherapy should be administered.

Conclusion

The last decade has seen major advances both in the surgical and medical management of renal cell cancer. Outstanding areas for further research include the use of adjuvant therapy, the role of surgery in metastatic disease in the era of TKIs, the synergistic combination of therapies, the sequential use of one TKI after the failure of another and novel immunotherapies. The optimal treatment of non-clear cell renal cancer is not yet well investigated and specific studies are underway in papillary carcinoma and in non-clear cell carcinoma more generally. The development of predictive and prognostic markers would be a major boon, especially given the great differences in behaviour between different renal cell cancers.

References

Benichou, J., Chow, W. H., McLaughlin, J. K., *et al.* (1998). Population attributable risk of renal cell cancer in Minnesota. *Am J Epidemiol*, 148(5), 424–30.

Bosniak, M. A. (1986). The current radiological approach to renal cysts. *Radiology*, 158(1), 1–10.

Breen, D. J., Rutherford, E. E., Stedman, B., *et al.* (2007). Management of renal tumors by image-guided radiofrequency ablation: experience in 105 tumors. *Cardiovasc Intervent Radiol*, 30(5), 936–42.

Chow, W. H., Dong, L. M., and Devesa, S. S. (2010). Epidemiology and risk factors for kidney cancer. *Nat Rev Urol*, 7(5), 245–57.

Cockman, M. E., Masson, N., Mole, D. R., *et al.* (2000). Hypoxia inducible factor-alpha binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. *J Biol Chem*, 275(33), 25733–41.

Edge, S. B., Byrd, D. R., Compton, C. C., *et al.* (eds.) (2010). Kidney. In *AJCC Cancer Staging Manual* (7th ed.), pp. 479–89. New York: Springer.

Eisenhauer, E. A., Therasse, P., Bogaerts, J., *et al.* (2009). New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*, 45(2), 228–47.

Escudier, B., Eisen, T., Stadler, W. M., *et al.* (2007a). Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med*, 356(2), 125–34.

Escudier, B., Pluzanska, A., Koralewski, P., *et al.* (2007b). Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. *Lancet*, 370(9605), 2103–11.

Gill, I. S., Kavoussi, L. R., Lane, B. R., *et al.* (2007). Comparison of 1,800 laparoscopic and open partial nephrectomies for single renal tumors. *J Urol*, 178(1), 41–6.

Gnarra, J. R., Tory, K., Weng, Y., *et al.* (1994). Mutations of the VHL tumour suppressor gene in renal carcinoma. *Nat Genet*, 7(1), 85–90.

Heng, D. Y., Xie, W., Regan, M. M., *et al.* (2009). Prognostic factors for overall survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor-targeted agents: results from a large, multicenter study. *J Clin Oncol*, 27(34), 5794–9.

Hudes, G., Carducci, M., Tomczak, P., *et al.* (2007). Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med*, 356(22), 2271–81.

Kavolius, J. P., Mastorakos, D. P., Pavlovich, C., *et al.* (1998). Resection of metastatic renal cell carcinoma. *J Clin Oncol*, 16(6), 2261–6.

Klapper, J. A., Downey, S. G., Smith, F. O., *et al.* (2008). High-dose interleukin-2 for the treatment of metastatic renal cell carcinoma: a retrospective analysis of response and survival in patients treated in the surgery branch at the National Cancer Institute between 1986 and 2006. *Cancer*, 113(2), 293–301.

Leibovich, B. C., Blute, M. L., Cheville, J. C., *et al.* (2003). Metastasis-free survival after RCC resection. *Cancer*, 97, 1663–71.

McLaughlin, J. K., Mandel, J. S., Blot, W. J., *et al.* (1984). A population-based case–control study of renal cell carcinoma. *J Natl Cancer Inst*, 72(2), 275–84.

Motzer, R. J., Mazumdar, M., Bacik, J., *et al.* (1999). Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. *J Clin Oncol*, 17(8), 2530–40.

Motzer, R. J., Hutson, T. E., Tomczak, P., *et al.* (2007). Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med*, 356(2), 115–24.

Motzer, R. J., Escudier, B., Oudard, S., *et al.* (2008). Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet*, 372(9637), 449–56.

Patard, J. J., Pantuck, A. J., Crepel, M., *et al.* (2007). Morbidity and clinical outcome of nephron-sparing surgery in relation to tumour size and indication. *Eur Urol*, 52(1), 148–54.

Rini, B. I., Halabi, S., Taylor, J., *et al.* (2004). Cancer and Leukemia Group B 90206: A randomized phase III trial of interferon-alpha or interferon-alpha plus anti-vascular endothelial growth factor antibody (bevacizumab) in metastatic renal cell carcinoma. *Clin Cancer Res*, 10(8), 2584–6.

Rini, B. I., Escudier, B., Tomczak, P., *et al.* (2011). Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial. *Lancet*, 378(9807), 1931–9.

Schmidinger, M., Zielinski, C. C., Vogl, U. M., *et al.* (2008). Cardiac toxicity of sunitinib and sorafenib in patients with metastatic renal cell carcinoma. *J Clin Oncol*, 26(32), 5204–12.

Sternberg, C. N., Davis, I. D., and Mardiak, J. (2010). Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol*, 28(6), 1061–8

Sunela, K. L., Kataja, M. J., and Kellokumpu-Lehtinen, P. L. (2010). Changes in symptoms of renal cell carcinoma over four decades. *BJU Int*, 106(5), 649–53.

Varela, I., Tarpey, P., Raine, K., *et al.* (2011). Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. *Nature*, 469(7331), 539–42.

CHAPTER 173

The patient with Wilms tumour

Christopher Mitchell

Introduction and definition

Wilms tumour (Wilms, 1899), the most common genitourinary malignancy of childhood, is a triphasic embryonal neoplasm consisting of varying proportions of blastema, stroma, and epithelium. Although Max Wilms described this specific histological appearance in 1899, the eponym is now loosely applied to virtually any malignant tumour arising in the kidney in childhood, although they are pathologically, clinically, and genetically distinct entities.

Epidemiology

The annual incidence of Wilms tumour is around eight per million children under the age of 15 years. There are both racial and regional variations in incidence, so that the previously held view that the incidence was constant throughout the world (the 'index' tumour) is not correct (Parkin et al., 1988). The risk of developing Wilms tumour is approximately 1 in 10,000 live births. The tumour accounts for about 8% of childhood malignancies so, in incidence, ranks fifth among the solid tumours of childhood, after tumours of the central nervous system, lymphoma, neuroblastoma, and soft-tissue sarcoma. The tumour occurs with equal frequency in boys and girls, with a peak incidence in the third year. It is very rare in the neonatal period (Hrabovsky et al., 1986); > 75% of children affected are < 4 years of age and at least 90% < 7 years at diagnosis (Breslow and Beckwith, 1982; see Fig. 173.1). Very few cases are diagnosed after the age of 11 years.

Presentation

Clinical features

Most children with Wilms tumour are well and present only because they have an abdominal mass detected by a parent or other person, although symptoms such as abdominal pain, haematuria, and fever may sometimes occur. Generally, the contrast with the clinical picture of abdominal neuroblastoma, the major differential diagnosis, is marked. Physical examination should include a search for evidence of the various conditions associated with WT1 mutations (see Chapter 329). These include hemihyperplasia (hemihypertrophy), Beckwith–Wiedemann syndrome, genital abnormalities, and aniridia. Hypertension, which may arise from excessive renin production, vascular compression by the tumour, or as part of pre-existing renal disease, occurs in a few patients and may be sufficiently severe to require treatment.

Abdominal examination reveals a smooth, rounded, or lobulated mass arising in the loin; it may be possible to feel the attached normal kidney. The mass is usually ballotable and does not move with respiration, thus allowing distinction from liver or spleen. The previously held view that abdominal examination should not be repeated for fear of tumour rupture or tumour emboli is probably false. Any metastases present at diagnosis, usually pulmonary, will only rarely be detected by clinical examination.

Investigations

The objectives of investigation are to confirm the diagnosis, delineate the extent of the tumour, determine that the contralateral kidney is functional, discover any metastases, and ensure that the child is fit enough to undergo anaesthesia and surgery.

A blood count may detect anaemia resulting from haemorrhage into the tumour; there may also be thrombocytosis in response to haemorrhage. A few patients develop a bleeding diathesis secondary to an acquired form of von Willebrand disease. An incidence of 8% was noted in one study (Coppes et al., 1992), so coagulation tests should be sent. Urinalysis, particularly for protein, and measurement of serum electrolytes, urea, and creatinine, should detect any gross abnormalities of renal function. Measurement of urinary catecholamines is essential to exclude neuroblastoma, especially in hypertensive children, and particularly if immediate surgery is contemplated. No imaging technique can exclude neuroblastoma with complete accuracy, and some are intrarenal. There are two reasons for taking care to exclude the diagnosis of neuroblastoma: first, immediate surgery would not be appropriate; and second, catecholamine-secreting tumours pose particular anaesthetic problems, which should be recognized preoperatively.

An abdominal ultrasound scan is the imaging investigation of choice for determining the organ of origin, the extent of any spread within the abdomen, the patency of the inferior vena cava, and for detecting any involved lymph nodes. Most centres would also perform a computed tomography (CT) or magnetic resonance (MR) scan of the abdomen to further define the anatomy of the tumour. It is important to document normal function of the contralateral kidney before any surgery is contemplated and the excretion of contrast at the end of a CT scan is useful confirmation of function in the contralateral kidney. A dimercaptosuccinic acid scan is an alternative investigation, and is particularly valuable in planning surgery for patients with bilateral tumours or with only a single functioning kidney where partial nephrectomy is contemplated.

A North American study found pulmonary disease by scanning but not by conventional radiography in 11 of 124 children (Willimas et al., 1988), but there was no significant difference in the relapse rate between that small group and the larger number of

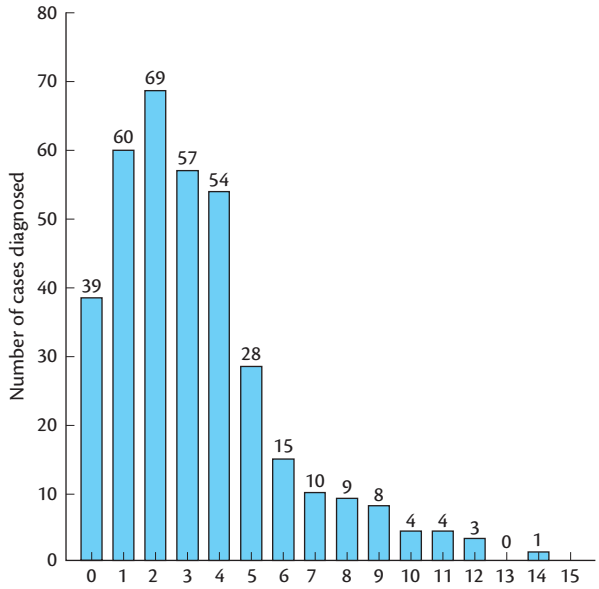

Fig. 173.1 Age-related number of newly diagnosed cases of Wilms tumour in children. (UK Child Cancer Study Group data from 361 cases, numbers shown at top of each bar).

patients whose lung lesions could be seen on ordinary radiography. The United Kingdom Children's Cancer Study Group (UKCCSG) reported a number of patients with metastases detected by CT that were not found by conventional radiography (31 out of 142), and found that just over 25% of these patients relapsed (7 out of 31). However, relapses were more common in patients who were otherwise stage I than those who were stages II to V (Mitchell et al., 2000). These data suggest that the majority of higher-stage patients will have their pulmonary disease adequately treated by their stage-appropriate chemotherapy and need not have their initial treatment intensified. Stage I patients, however, receive so little additional therapy that great care is needed in defining the stage and it is here that CT scanning may be important.

Postoperatively, other imaging investigations may be indicated by specific histological findings. A ^{99}Tc bone scan is indicated after the diagnosis of the so-called bone-metastasizing renal tumour (also called clear cell sarcoma; see below). An additional radiological survey of the skeleton is unnecessary. In malignant rhabdoid tumour of the kidney (RTK) an MR scan of the head is needed to exclude the presence of an associated intracranial tumour (Bonnin et al., 1984).

Prognostic features

Prognostic factors arise as artefacts of imperfect treatment regimens. Thus, if treatment was uniformly successful, or a disease uniformly fatal, there would be no prognostic factors. The early recognition of prognostic factors in Wilms tumour has permitted stratification of treatment, and so directed intensive treatment to patients with 'bad risk' disease and reduced treatment to patients with 'good risk' disease. The standard prognostic factors include pathology and surgical stage, both of which reflect underlying biological processes and the latter to some extent also reflecting the length of time before diagnosis and, possibly, surgical skill. Subsequently, response to initial chemotherapy, as delineated by SIOP (Societe Internationale D'Oncologie Pediatrique) studies has developed as an additional factor, and more recently the prognostic significance of a number of molecular genetic factors has been noted. In the future, use of biological markers combined with histological response to preoperative chemotherapy seems likely to provide the most useful risk adapted system of therapy, as has already been seen, for example, in the therapy of acute lymphoblastic leukaemia in childhood.

Pathology

Two broad groups of tumours may be recognized by their histological appearances (for review, see Beckwith, 1986). The majority have classical triphasic tumours, in which epithelial, blastemal, and stromal elements are all present. Some triphasic tumours may have rhabdomyoblastic differentiation, such that the cells resemble fetal rhabdomyoblasts, often with cross-striations. This appearance is not unfavourable and it must not be confused with the 'malignant rhabdoid tumour of the kidney', which is a variant with poor prognosis (see below).

The monomorphic epithelial variant, usually found in children < 1 year of age, is easily recognized as it appears to consist entirely of primitive tubules. This appearance has a very favourable prognosis, as do stage I tumours weighing < 550 g in patients < 2 years of age (Green et al., 1994). Both of these subtypes are effectively treated by nephrectomy only.

The second group of patients have tumours that are categorized as one of the unfavourable histologies. Anaplasia is an unfavourable feature occasionally observed in triphasic tumours, where it is characterized by large (greater than four times normal) hyperchromatic nuclei, an increased nuclear:cytoplasmic ratio, and abnormal (e.g. tripolar) mitoses. Anaplasia in Wilms tumour is often a patchy, focal change, which may escape notice unless a deliberate search is made; including widespread sampling with blocks cut every centimetre across the widest diameter of the tumour. The appearances are often best recognized by scanning the slide at low power.

The other unfavourable histological types are distinct tumours, rather than true variants of Wilms tumour. The bone-metastasizing renal tumour of childhood, or clear cell sarcoma of the kidney (CCSK), was first reported by Kidd (1970), and it was later separately identified by Marsden and Lawler (1978) from the United Kingdom, and by Beckwith and Palmer (1978) from America: they all describe a distinctive neoplasm with a propensity for skeletal metastasis and aggressive clinical behaviour. The incidence of reported bone metastases was 76% of 38 cases in the British series and 17% of 75 cases in the American series.

In the third National Wilms Tumour Study, the bone-metastasizing tumour formed nearly 6% of all cases, making it the most frequent form of 'unfavourable' histology. Its age distribution is similar to that of Wilms tumour. There appears to be a distinct male preponderance for this type, both in American and British series, although not as great as originally suggested.

Malignant RTK, recognized by Beckwith and Palmer (1978) in their report from the first National Wilms Tumour Study is a very unfavourable tumour. It is the least common of the unfavourable entities and was found in only 2% of patients entered in the National (American) studies. The age distribution is markedly different to that of Wilms tumour, with nearly half the patients being diagnosed in the first year of life (for review, see Weeks et al.,

1989). It is also associated with other primary tumours of various types (usually primitive neuroectodermal tumours) arising in the posterior intracranial fossa (Bonnin et al., 1984). The intracranial tumour may precede or follow the renal tumour. Hypercalcaemia has been reported in a number of cases of malignant rhabdoid tumour (Rousseau-Merck et al., 1982; Mitchell et al., 1985), but may also occur in congenital mesoblastic nephroma.

The genetic basis of RTK indicates that it is a distinct entity from classical Wilms tumour. Cytogenetic, fluorescence *in situ* hybridization, and loss-of-heterozygosity (LOH) studies have revealed that malignant rhabdoid tumours frequently contain deletions at chromosome locus 22q11, which contains the *SWI/SNF* related, matrix-associated, actin-dependent regulator of chromatin, subfamily B, member 1 (*SMARCB1*) gene, also known as human sucrose non-fermenting gene number 5 (*hSNF5*).

Two distinct, low-risk entities have been described. The first is congenital mesoblastic nephroma, which is a rare, distinctive tumour of the infantile kidney (Bolande, 1973). There have been reports of local recurrences and metastases but in only one of these was the patient < 3 months of age. Review of the specimen showed that tumour extended to the margin of resection. There have been other recurrences in patients > 3 months of age; the microscopic appearances were of dense cellularity and numerous mitotic figures. Vascular invasion and tumour rupture have also been associated with an unfavourable outcome. The other low-risk entity is cystic partly differentiated nephroblastoma (CPDN), which is usually seen in children under the age of 2 years. The tumour is composed entirely of cysts and their thin walls, with the septa forming the only 'solid' component of the tumour. The cysts are lined by cuboidal epithelium and the septa contain blastema together with stroma or epithelium. The tumour forms a discrete mass, well demarcated from the non-cystic renal parenchyma. Surgery alone is usually adequate therapy for both of these tumours.

It has been demonstrated that chemotherapy induced changes are prognostically important, as one might expect (Zuppan et al., 1991; Boccon-Gibod et al., 2000). In the SIOP risk assessment system, use is made of the response to a standard preoperative chemotherapy regimen, which now consists of four doses of vincristine at weekly intervals with the addition of actinomycin D at weeks 1 and 3. Low risk tumours, as noted above, include mesoblastic nephroma, CPDN, and in addition, completely necrotic nephroblastoma, with 100% survival regardless of 'stage' and hence with no further therapy necessary after resection. The histological criteria for making this diagnosis are the absence of any viable tumour tissue on gross or microscopic examination of multiple blocks, and the presence of regressive or necrotic changes caused by the chemotherapy.

Designating intermediate-risk tumours involves first assessing the percentage of necrosis or regression that is present. If these changes are present in > 66% of the tumour, it may then be designated as being of regressive subtype. If the degree of change is < 66% the tumour may be subtyped according to the predominant histological component, or designated as mixed if there is no predominance. Five entities may be recognized: epithelial, stromal, mixed, regressive, or with focal anaplasia. Each of these entities is of intermediate prognosis.

Conversely, the persistence of blastema after chemotherapy indicates chemoresistance and has been shown to be associated with a poor outcome (Weirich et al., 2001). In order to make a diagnosis of blastemal type nephroblastoma, the viable component must be > 33% of the tumour mass and at least 66% of the viable areas must be blastema. Other components may also be present in varying amounts. Other high-risk entities include diffuse anaplasia, CCSK and malignant RTK.

Surgical staging systems

Several staging systems have been used for Wilms tumour, evolving as successive studies have redefined the criteria for each stage. The major contribution to these systems has been from the North American National studies. This staging system has been used subsequently in National Wilms Tumor Study Group (NWTS) studies 4 and 5 and also in the UK Wilm's (UKW) series of studies 1 and 2, with adaptions in UKW3.

The use of preoperative chemotherapy obviously has a major impact on the validity of the NWTS staging system. The SIOP group use a combination of adapted NWTS staging and pathological appearances to incorporate the implications of early disease response to chemotherapy, to categorize patients into risk groups.

For details of staging methods, see Further Reading.

Molecular genetics

Wilms tumour gives its name to the *WT1* (Wilms tumour 1) gene (see Chapter 329) which is located at chromosome 11p13. *WT1* is a transcription factor with complex effects, and a classic tumour suppressor gene. The incidence of Wilms tumours is increased in those with germline WT1 mutations, but it is mutated in only a minority (probably 10–15%) of sporadic cases of the tumour. Other implicated genes include *CTNNB1*, encoding β-catenin, and *WTX* (*AMER1*). Again these genes are found in < 20% of Wilms tumours, but mutations may occur together. A defect of 11p15 (the WT2 locus) is particularly associated with Beckwith-Wiedermann Syndrome, and these individuals are more likely to develop bilateral tumours. Epigenetic changes may be important but germline mutations at this locus are found in < 3% of patients (Scott et al. 2008). Other genes are implicated with a still lower frequency. For a review of the molecular genetics of the tumour see Hohenstein et al. (2015).

In a study of 232 patients registered in NWTS-3 and -4, Grundy et al. (1994) found that LOH of markers on 16q, found in 17.2% of patients with favourable histology or anaplasia, had a significantly lower relapse-free survival and overall survival (OS), even after allowing for stage or histology. LOH for markers at region 1p was found in 11% of the samples, and again was associated with poorer relapse-free survival and OS. LOH at 11p or duplication of 1q were not associated with any difference in outcome.

Prospective assessment of these markers in NWTS-5 confirmed that the phenomenon of LOH at the 1p and 16q regions was indeed an adverse factor. Prospective assessment of biological factors by both the NWTS and SIOP groups and has been demonstrated to identify a group of patients at higher risk of recurrence; LOH for markers on the long arm of chromosome 16 and the short arm of chromosome 1 (16q and 1p) are associated with a worse outcome. In NWTS-5, stage I and II patients without LOH at these loci had a survival of 91.2% versus only 74.9% of patients with LOH (Grundy et al., 2005).

Treatment

Despite advances in chemotherapy, surgical resection is, and almost certainly will remain, the fundamental treatment for Wilms tumour.

There remains, however, debate about the timing of surgical intervention, the place of percutaneous needle biopsy, and the use of preoperative chemotherapy. North American practice remains steadfastly in favour of immediate surgery followed by adjuvant therapy dictated by the surgical stage. In contrast, the SIOP group in Europe conducted a series of trials based on the use of preoperative therapy—but conflicting recommendations remain (summarized in Bhatnagar, 2009; Graf et al., 2000; Green, 2004; Vujanić, 2006).

Radiotherapy is usually reserved for Stage 3+ disease. Chemotherapy is generally based on dactinomycin, vincristine, and doxorubicin. Detailed consideration of treatment is beyond the scope of this text, but see Further Reading for additional information and references.

Long-term complications of therapy

The long term implications of childhood treatment for cancer are increasingly being recognised. These include the effects of chemotherapeutic agents, including effects on kidney function (see Chapter 362).

It is often assumed that unilateral nephrectomy has only minimal, if any, long-term morbidity, as usually expected for congenital solitary kidney (see Chapter 351). However kidneys that develop Wilms tumour may not be normal if there was a predisposing mutation or developmental abnormality, and the surviving kidney may have been exposed to radiation (see Chapter 91). Cytotoxic drugs commonly used for Wilms tumour are not usually thought to be nephrotoxic. An evaluation of renal function in a group of long-term Wilms tumour survivors (> 13 years off treatment) has shown some evidence of dysfunction of the remaining kidney in 32%. Nineteen per cent had measured glomerular filtration rates of < 80 mL/min/1.73m^2, 11% had hypertension, and 9% had increased urinary albumin excretion. Only 55% of patients had undergone significant compensatory contralateral renal hypertrophy. Children aged < 24 months at the time of diagnosis and those children who had received radiation doses of > 1200 cGy to the remaining kidney were most at risk of dysfunction (Levitt et al., 1992). A similar study in adults with solitary kidneys who had undergone partial nephrectomy for a renal malignancy concluded that there was an increased risk of proteinuria, glomerulopathy, and progressive renal failure (Novick et al., 1991). Thus, there is a clear case for continuing follow-up with measurements of blood pressure and checks for proteinuria.

The major long-term side effect of radiotherapy for Wilms tumour is disturbance of growth. As the radiation field in virtually all children irradiated for this condition is the hemiabdomen, the most obvious disturbance is asymmetrical growth of soft tissue. As the field includes the full width of the vertebral bodies, there is also a loss in final height. The younger the patient is at the time of irradiation the more severe is the restriction and the more disproportionate they become as adults. The loss in potential height ranges from 10 cm at the age of 1 year to 7 cm at 5 years (Wallace et al., 1990).

Prognosis

The majority of patients with Wilms tumour will be cured. In part this is because most patients will present with histologically favourable, low-stage disease, but there have also been genuine advances in chemotherapy, so that the vast majority of Wilms tumour patients are cured, many with minimal short- or long-term morbidity. Overall 5 year survival rates are now over 90% (Smith et al., 2014). Poor outcomes are concentrated in patients with highest stage disease.

Further reading

National Cancer Institute (updated 8 July 2015) Wilms Tumor and Other Childhood Kidney Tumors Treatment–for health professionals (PDQ®) http://www.cancer.gov/types/kidney/hp/wilms-treatment-pdq Accessed 1 Aug 2015.

Israles, T., Moreira, C. l, Scanlan, T., *et al.* (2012). SIOP PODC: Clinical guidelines for the management of children with Wilms tumour in a low income setting. *Ped Blood Cancer* 60, 5–11.

Dome, J. S., Huff, V. (update Sep 2013). Wilms tumor overview. Gene Reviews (Pagon RA, Adam MP, Ardinger HH, *et al.*, eds.) Seattle (WA): University of Washington, Seattle; 1993–2015. http://www.ncbi.nlm.nih.gov/books/NBK1294

Human, C., Beckwith, J. B., Smith, A. C., Weksberg, R. (updated 2010). Beckwith-Wiedemann Syndrome. Gene Reviews (Pagon RA, Adam MP, Ardinger HH, *et al.*, eds.) Seattle (WA): University of Washington, Seattle; 1993–2015. http://www.ncbi.nlm.nih.gov/books/NBK1394

References

Beckwith, J. B. (1986). Wilms' tumour and other renal tumors of childhood. In M. W. B. Finegold (ed.) *Pathology of Neoplasia in Children and Adolescents*, pp. 313–32. Philadelphia, PA: Saunders.

Beckwith, J. B. and Palmer, N. F. (1978). Histopathology and prognosis of Wilms' tumour: results from the first National Wilms' tumour study. *Cancer*, 41, 1937–48.

Bhatnagar, S. (2009). Management of Wilms' tumor: NWTS vs SIOP. *J Indian Assoc Pediatr Surg*, 14, 6–14. doi: 10.4103/0971-9261.54811 PMCID: PMC2809467

Boccon-Gibod, L., Rey, A., Sandstedt, B., *et al.* (2000). Complete necrosis induced by preoperative chemotherapy in Wilms' tumor as an indicator of low risk: report of the International Society of Paediatric Oncology (SIOP) Nephroblastoma Trial and Study 9. *Med Pediatr Oncol*, 34, 193–200.

Bolande, R. P. (1973). Congenital mesoblastic nephroma of infancy. *Perspect Pediatr Pathol*, 1, 227–50.

Bonnin, J. M., Rubinstein, L. J., Palmer, N. F., *et al.* (1984). The association of embryonal tumours originating in the kidney and in the brain. A report of seven cases. *Cancer*, 54, 2137–6.

Breslow, N. E. and Beckwith, J. B. (1982). Epidemiological features of Wilms' tumour: results of the National Wilms' Tumour Study. *J Natl Cancer Inst*, 68, 429–36.

Coppes, M. J., Zandvoort, S. W., Sparling, C. R., *et al.* (1992). Acquired von Willebrand disease in Wilms' tumour patients. *J Clin Oncol*, 10, 422–7.

Graf, N., Tournade, M. F., de Kraker, J. (2000). The role of preoperative chemotherapy in the management of Wilms' tumor. The SIOP studies. International Society of Pediatric Oncology. *Urol Clin North Am*, 27, 443–454.

Green, D. M. (2004). The treatment of stages I–IV favorable histology Wilms' tumor. *J Clin Oncol.* 22, 1366–1372.

Green, D. M., Beckwith, J. B., Weeks, N. M., *et al.* (1994). The relationship between microstaging variables, age at diagnosis, and tumor weight of children with stage I/favorable histology Wilms' tumor. *Cancer*, 74, 1817–20.

Grundy, P. E., Telzerow, P. E., Breslow, N., *et al.* (1994). Loss of heterozygosity for chromosomes 16q and 1p in Wilms' tumors predicts an adverse outcome. *Cancer Res*, 54, 2331–3.

Grundy, P. E., Breslow, N. E., Li, S., *et al.* (2005). Loss of heterozygosity for chromosomes 1p and 16q is an adverse prognostic factor in favorable-histology Wilms tumor: a report from the National Wilms Tumor Study Group. *J Clin Oncol*, 23, 7312–7321.

Hohenstein, P., Pritchard-Jones, K., and Charlton, J. (2015). The yin and yang of kidney development and Wilm's tumors. *Genes Dev*, 29, 467–82.

Hrabovsky, E. E., Othrsen, H. B., deLorimier, A., *et al.* (1986). Wilms' tumour in the neonate: a report from the national Wilms' tumour study. *J Pediatr Surg*, 21, 385–7.

Kidd, J. M. (1970). Exclusion of certain renal neoplasms from the category of Wilms' tumour. *Am J Pathol*, 59, 16a.

Levitt, G. A., Yeomans, E., Dicks-Mireaux C., *et al.* (1992). Renal size and function after cure of Wilms' tumour. *Br J Cancer*, 66, 877–82.

Marsden, H. B. and Lawler, W. (1978). Bone-metastasising renal tumour of childhood. *Br J Cancer*, 38, 437–41.

Mitchell, C. D., Harvey, W., Gordon, D., *et al.* (1985). Rhabdoid Wilms' tumour and prostaglandin-mediated hypercalcaemia. *Eur Paediatr Haematol Oncol*, 2, 153–7.

Mitchell, C. D., Morris-Jones, P., Kelsey, A., *et al.* (2000). The treatment of Wilms' tumour: results of the UKCCSG second Wilms' tumour study. *Brit J Cancer*, 83, 602–8.

Novick, C., Gephardt, M. D., Guz, B., *et al.* (1991). Long-term follow-up after partial removal of a solitary kidney. *N Engl J Med*, 325, 1058–62.

Parkin, D. M., Stiller, C. A., and Draper, G. J. (1988). The international incidence of childhood cancer. *Int J Cancer*, 42, 511–20.

Rousseau-Merck, M. F., Boccon-Gibod, L., Nogues, C., *et al.* (1982). An original hypercalcemic infantile renal tumour without bone metastasis: heterotransplantation to nude mice. *Cancer*, 50, 85–93.

Scott, R. H., Douglas, J., Baskcomb, L., *et al.* (2008). Constitutional 11p15 abnormalities, including heritable imprinting center mutations, cause nonsyndromic Wilms tumor. *Nat Genet*, 40, 1329–34.

SIGN (2013). Long term follow up of survivors of childhood cancer. A national clinical guideline. Edinburgh: SIGN, publication number 132. Available from http://www.sign.ac.uk

Smith, M. A., Altekruse, S. F., Adamson, P. C., *et al.* (2014). Declining childhood and adolescent cancer mortality. *Cancer*, 120, 2497–506.

Vujanić, G. M., Kelsey, A., Mitchell, C., *et al.* (2006). The role of biopsy in the diagnosis of renal tumors of childhood: Results of the UKCCSG Wilms tumor study 3. *Med Pediatr Oncol*, 40, 18–22.

Wallace, W. H., Shalet, S. M., Morris-Jones, P. H., *et al.* (1990). Effect of abdominal irradiation on growth in boys treated for a Wilms' tumor. *Med Pediatr Oncol*, 18, 441–6.

Weeks, D. A., Beckwith, J. B., Merau, G. W., *et al.* (1989). Rhabdoid tumour of the kidney. A report of 111 cases from the National Wilms' Tumour Study center. *Am J Surg Pathol*, 13, 439–58.

Weirich, A., Leuschner, I., Harms, D., *et al.* (2001). Clinical impact of histologic subtypes in localised non anaplastic nephroblastoma treated according to trial and study SIOP9/GPOH. *Ann Oncol*, 12, 311–19.

Wiedemann, N. R. (1964). Complexe malformatif familial avec hernie ombilicale et macroglossie: un syndrome nouveau? *J Genet Hum*, 13, 223–32.

Wilimas, J. A., Douglass, E. C., Magill, H. L., *et al.* (1988). Significance of pulmonary computed tomography at diagnosis in Wilms' tumour. *J Clin Oncol*, 6, 1144–6.

Wilms, M. (1899). *Die Mischgeschwulste der Nieren*. Leipzig: Arthur Georgi.

Zuppan, C. W., Beckwith, J. B., Weeks, D. A., *et al.* (1991). The effects of preoperative chemotherapy on the histologic features of Wilms' tumor. An analysis of cases from the third national Wilms' Tumor Study. *Cancer*, 68, 385–94.

CHAPTER 174

The patient with haemolytic uraemic syndrome/thrombotic thrombocytopenic purpura

Marina Noris and Tim Goodship

Introduction

The patient who presents with microangiopathic haemolytic anaemia, thrombocytopenia, and evidence of acute kidney injury can be a diagnostic and management challenge. Haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are two of the conditions that frequently present with this triad (Table 174.1).

The term HUS was first used by the Swiss haematologist Conrad Von Gasser in a paper published in 1955 (Gasser et al., 1955). In this paper, Von Gasser described five patients with renal failure, thrombocytopenia, and acquired haemolytic anaemia. Since then HUS has mainly been used in association with a thrombotic microangiopathy occurring following infection with verocytotoxin (Shiga-like toxin) producing bacteria, particularly enterohaemorrhagic *Escherichia coli* (STEC). Most individuals with this condition (STEC-HUS) recover renal function. Less often, patients present with the features of HUS without any evidence of infection with verocytotoxin-producing bacteria. In this group, the prognosis is worse with a significant number developing end-stage renal failure. Over time, the prefix 'atypical' has been added to define this group.

The term TTP was first used by Singer et al. in a paper that was published in *Blood* in 1947 (Singer et al., 1947). They described one case of their own and identified 11 previously reported cases which were characterized by:

- 'Petechiae and ecchymoses, thrombocytopenia, prolonged bleeding time and poor clot retraction'
- 'Mild acholuric jaundice, hepatosplenomegaly'
- 'Bizarre and intermittent mental and neurologic symptoms and signs'
- 'A transient leukemoid reaction in the peripheral blood.'

They stated that 'this clinical picture must be correlated with a remarkable histologic pattern, namely the presence of myriads of platelet thrombi in the small arterioles and capillaries of almost all organs of the body'. Of the 11 previously reported cases included in this paper, the first was from Eli Moschowitz. In 1925, he reported a case in a16-year-old female which as the title of paper states was characterized by 'an acute febrile pleiochromic anaemia with hyaline thrombosis of the terminal arterioles and capillaries' (Moschcowitz, 1925). This in retrospect was recognized to be the first description of what 22 years later became known as TTP.

The past two decades have seen a remarkable increase in our understanding of the molecular mechanisms underlying these conditions. The nomenclature used to describe diseases characterized by a thrombotic microangiopathy has been a source of debate for decades. Defining the molecular mechanisms underlying these conditions in the past two decades enabled the development of a mechanistic classification (Besbas et al., 2006).

Shiga toxin-producing *Escherichia coli*-associated haemolytic uraemic syndrome

Epidemiology

HUS is rare with an overall incidence of 0.2–1 case for 100,000 persons/year, with a peak of 5–6 per 100,000/year in children under the age of 5 years (Noris and Remuzzi, 2009). Most cases (> 90% of those in children) are associated with infection by strains of *Escherichia coli* that produce potent cytotoxins, the Shiga-like toxins (Stxs) (Table 174.1). The best known Stx-producing *Escherichia coli* (STEC) strain is O157:H7 (Mead and Griffin, 1998), but infections sustained by STEC strains belonging to serogroups other than O157, like O26, O111, O103, O123, O145, and the O104:H4 strain isolated in the recent German outbreak, have been increasingly reported (Caprioli et al., 2005; Frank et al., 2011). High rates of HUS have been reported in regions of South America, especially Argentina, where HUS is endemic with an incidence 5–10 times higher than in North America. In Argentina, STEC-HUS is the main cause of acute kidney injury in children and the second commonest cause of chronic renal failure, accounting for 20% of renal transplants in children and adolescents (Palermo et al., 2009).

STEC-HUS occurs primarily in children except in epidemics when it may occur in patients with a wider range of ages. For example, from May 2011 until July 2011, several European Countries, particularly Northern Germany, experienced one of the largest STEC-HUS outbreaks ever reported with 3816 patients suffering from *E. coli* O104:H4 infection, with 845 HUS cases. Almost 90% of affected patients were adults and, compared to previous STEC

Table 174.1 Classification of haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) according to clinical presentation and underlying aetiology

Clinical presentation		Aetiology
Haemolytic uraemic syndrome		
STEC-associated		Infections by Stx-producing bacteria
Neuraminidase associated		Infections by *Streptococcus pneumoniae*
Atypical		*Mutations: CFH*, 30%; *CFI*, 5–10%; *C3*, 8–10%; *MCP*, 10–15%; *THBD*, 4%; *CFB*, 1–2%.
		Anti-CFH autoantibodies: 5–10%
Thrombotic thrombocytopenic purpura		
Congenital		Homozygous or compound heterozygous ADAMTS13 mutations
Idiopathic		Anti-ADAMTS13 autoantibodies
Secondary	Ticlopidine clopidogrel	Anti-ADAMTS13 autoantibodies (ticlopidine: 80–90%, clopidogrel: 30%)
	HSC transplantation	Unknown, rarely low ADAMTS13
	Malignancies	Unknown, rarely low ADAMTS13
	HIV	HIV virus, rarely low ADAMTS13
	SLE, APL, and other autoimmune disease	Depends on the primary diseases

APL = antiphospholipid syndrome; HSC = haematopoietic stem cell transplantation; SLE = systemic lupus erythematosus; STEC = Shiga-like toxin producing *E.coli*; Stx = Shiga-like toxin.

epidemics, there was a higher prevalence of affected young and middle-aged women (Frank et al., 2011).

Infection with STEC is more common in the warm summer months in both the northern and southern hemispheres. The consistency of this finding, together with the observation that STEC shedding by animals is also seasonal (Mead and Griffin, 1998), suggests that climatic factors play an important role in determining the incidence of human infection.

Healthy cattle are a major reservoir for human infection with STEC. Environmental studies have shown that the organism can persist in manure, water troughs, and other places on farms (Mead and Griffin, 1998). In addition to cattle, the bacteria have been isolated from deer, sheep, goats, horses, dogs, birds, and flies (Chapman et al., 1997; Caprioli et al., 2005). Shedding by ruminants is particularly common, suggesting these animals provide a specific niche for the bacterium. Although most human infections occur in urban areas, people living in rural areas may be at greater risk of infection, presumably because of greater exposure to livestock (MacDonald et al., 1996).

STEC is transmitted by food and water, directly from one person to another, and occasionally through occupational exposure. Most food-borne outbreaks have been traced to food derived from cattle, especially ground beef and raw milk (Mead and Griffin, 1998). Meat probably becomes contaminated at the time of slaughter and grinding may compound the problem by introducing the pathogen into the interior of the meat, where it is more likely to survive cooking.

In Argentina, the high incidence of STEC-HUS has been associated with the extensive cattle farming and the high consumption of meat. About 20% of Argentine children start to eat meat at 5 months old, and 80% of them have meat in their diets at least three times a week. Eighty per cent of the meat consumed is undercooked (Rivas et al., 2006).

In the past few years, fruits and vegetables have accounted for a growing number of recognized STEC infections. Radish sprouts have been implicated in several O157:H7 outbreaks in Japan (Mead and Griffin, 1998). In the United States, fresh produce such as lettuce, apple cider, unpasteurized apple juice, and alfalfa sprouts have been implicated. A widespread outbreak associated with spinach in North America had dramatically higher than typical rates of both hospitalization (52%) and HUS (16%), due to the emergence of a new variant of O157:H7 serotype that has acquired several gene mutations that likely contributed to more severe disease (Manning et al., 2008). While some produce-associated outbreaks may be due to cross-contamination from meat products, others are more likely to reflect direct contamination in the field with faeces of wild or domestic animals (Centers for Disease Control and Prevention, 1997). Sprouts may pose a special hazard since pathogens present in trace amounts in seeds may multiply during sprouting. The consumption of sprouts was identified as the most likely vehicle of infection in the large recent German O104:H4 epidemic in Germany (Buchholz et al., 2011; Frank et al., 2011). The chain of transmission appeared to have started in Egypt with faecal contamination of fenugreek seeds by either humans or farm animals. During sprout germination, bacteria multiplied and produced large amounts of toxin and were then diffused with food provided to restaurants and consumers. It has been suggested that the higher

prevalence of women in this outbreak reflects a gender-specific dietary preference (Frank et al., 2011).

Waterborne outbreaks of STEC O157 infection have occurred as a result of drinking and swimming in unchlorinated water (Akashi et al., 1994; Keene et al., 1994). Person-to-person transmission occurs in day-care and chronic care facilities; settings that combine a high potential for transmission with a population at increased risk for severe outcomes (Mead and Griffin, 1998).

In humans, STEC may be shed in the stools for several weeks following resolution of symptoms. In general, young children carry the organism longer than do older children or adults. In one study, the median duration of faecal shedding among children < 5 years old was 17 days after onset of symptoms (Belongia et al., 1993).

Clinical features

Following exposure to STEC, 38–61% of individuals develop haemorrhagic colitis and 3–9% (in sporadic infections) to 20% (in epidemic forms) progress to overt HUS (Mead and Griffin, 1998; Banatvala et al., 2001). STEC-induced haemorrhagic colitis not complicated by HUS is self-limiting and is not associated with an increased long-term risk of high blood pressure or renal dysfunction, as shown by a 4-year follow-up study in 951 children who were exposed to a drinking water outbreak of *E. coli* 0157:H7 (Garg et al., 2006).

STEC-HUS is defined as the triad of haemolytic anaemia with erythrocyte fragmentation, thrombocytopenia, and acute kidney injury (Garg et al., 2009) that occurs after a prodromal infection by a STEC strain. The average interval between *E. coli* exposure and illness is 3 days. Illness typically begins with abdominal cramps and non-bloody diarrhoea; diarrhoea may become haemorrhagic in 70% of cases, usually within 1 or 2 days (Mead and Griffin, 1998). Vomiting occurs in 30–60% of cases and fever in 30%. Leucocyte count is usually elevated, and a barium enema may demonstrate 'thumb-printing', suggestive of oedema and submucosal haemorrhage, especially in the region of the ascending and transverse colon. HUS is usually diagnosed 6–10 days after the onset of diarrhoea (Mead and Griffin, 1998). After infection, STEC may be shed in the stools for several weeks after the symptoms are resolved, particularly in children < 5 years of age (Ruggenenti et al., 2001). Bloody diarrhoea, fever, vomiting, elevated leucocyte count, extremes of age, and female sex, as well as the use of antimotility agents, have been associated with an increased risk of HUS following an *E. coli* infection (Mead and Griffin, 1998; Beatty et al., 2004).

STEC-HUS is not a benign disease. Seventy per cent of patients require red blood cell transfusion, and 40–50% of cases require dialysis for an average duration of approximately 10 days, while the remainder have milder renal involvement without the need for dialysis (Milford, 1992; Mead and Griffin, 1998; Garg et al., 2003). About 25% of STEC-HUS patients have neurological involvement, including lethargy, apnoea, cortical blindness, hemiparesis, stroke, seizure, and coma (Tarr et al., 2005). There is evidence that the frequency and severity of neurological complications may be related to the intensity of the antecedent enteritis, evidenced by the degree of bloody diarrhoea and gastrointestinal symptoms (Siegler, 1995). Rare complications include pancreatitis, diabetes mellitus, and pleural and pericardial effusions.

Although mortality for infants and young children in industrialized countries decreased with the introduction of dialysis and intensive care facilities, 1–2% of patients still die during the acute phase of STEC-HUS (Milford, 1992)

Disease presentation and outcome were particularly severe during the STEC O104:H4 German outbreak, in which 50 of the 845 HUS cases in Germany died and 15 in other countries by 20 July 2011 (Blaser, 2011). Compared to previous STEC epidemics there was a higher incidence of dialysis-dependant kidney failure (20% vs 6%) and death (6% vs 1%) (Frank et al., 2011). Near half of the patients presented neurological symptoms that occurred on average 5.3 days after the onset of diarrhoea and 4 days after onset of HUS. Of the patients with neurological symptoms, 67% presented with cognitive impairment and aphasia. During the course of the disease, 20% of the patients suffered seizures. The onset of neurological symptoms was paralleled by increases in blood urea nitrogen and serum creatinine. Magnetic resonance imaging in these patients showed symmetrical hyperintense areas in the region of abducens nucleus and lateral thalamus. On follow-up scans, these abnormalities had resolved (Magnus et al., 2012).

The severe clinical phenotype seen in the German epidemic was in part explained by lack of previous immunity to this novel STEC strain and also by the exceptional virulence of the strain (Ruggenenti and Remuzzi, 2011). *E. coli* O104:H4 not only produces the same Shiga toxin as STEC enterohaemorrhagic strains (Qin et al., 2011) but also has 93% of the genomic sequence of enteroaggregative *E. coli*. These strains of *E.coli* form fimbriae which facilitate adhesion to the intestinal wall. The evolution of *E.coli* O104:H4 is likely the result of the acquisition by an enteroaggregative strain of *E.coli* of a Shiga toxin-encoding phage from a Shiga toxin-producing enterohaemorrhagic strain of *E.coli*. The combination of these two virulence factors would lead to increased gut colonization and thus the release of increased quantities of toxin into the circulation. Moreover, while enterohaemorrhagic *E. coli* are found in the gastrointestinal tract of ruminants, enteroaggregative *E. coli* have adapted to the human gut and appear to have their reservoir in humans (Qin et al., 2011). This might explain why this strain has acquired new resistances to antibiotics most commonly used in human disease that are in large part mediated by extended-spectrum beta-lactamases (ESBLs) (Frank et al., 2011).

Investigations

Detection of STEC and Stx

Diagnosis depends on the detection of *E.coli* O157:H7 and other Stx-producing bacteria and their products in stool cultures. When infection with a STEC is suspected, physicians should ensure that stool specimens are collected promptly and specifically cultured for the organism. In one study, detection of *E. coli* O157 in cultures declined from > 90% for stools collected during the first 6 days of illness to 33% for stools collected later (Tarr et al., 1990). Unlike most other *E. coli*, serotype O157:H7 does not ferment sorbitol rapidly and thus forms colourless colonies on sorbitol containing MacConkey agar (SMAC).The use of SMAC provides a simple, inexpensive and generally reliable method of screening stools for *E. coli* O157. Suspect colonies can be assayed for the O157 antigen with commercially available antiserum or latex agglutination kits. Newer protocols that use SMAC that contains cefixime tellurite, other selective culture media, immunomagnetic separation, and enzyme-linked immunosorbent assays to detect O157 lipopolysaccharide or Shiga toxins can further enhance detection (Mead

and Griffin, 1998). Unfortunately, none of the major non-O157 serotypes has a known biochemical marker, such as the lack of sorbitol fermentation, to facilitate screening in the clinical laboratory. The use of tests that identify Shiga toxins or the genes encoding them (by polymerase chain reaction) is helpful for diagnosis. Convalescent-phase serum samples can be assayed for antibodies to O157 or other specific strain-derived lipopolysaccharide (LPS), although this test is not commercially available and does not provide a diagnosis acutely (Mead and Griffin, 1998).

Laboratory findings

Laboratory features of thrombocytopenia and microangiopathic haemolytic anaemia are almost invariably present in patients with STEC-HUS and reflect consumption and disruption of platelets and erythrocytes in the microvasculature (Ruggenenti et al., 2001; Noris and Remuzzi 2009). Haemoglobin levels are low (haemoglobin ≤ 10 g/100 mL). Reticulocyte counts are uniformly elevated. The peripheral smear reveals increased schistocyte number, with polychromasia and, often, nucleated red blood cells. The latter may represent not only a compensatory response, but also damage to the bone marrow–blood barrier resulting from intramedullary vascular occlusion. Detection of fragmented erythrocytes is crucial to confirm the microangiopathic nature of the haemolytic anaemia. Other indicators of intravascular haemolysis include elevated lactate dehydrogenase (LDH), increased indirect bilirubin, and low haptoglobin levels (Ruggenenti et al., 2001; Noris and Remuzzi, 2009). The Coombs test is negative. Moderate leucocytosis often accompanies the haemolytic anaemia. Thrombocytopenia is uniformly present (platelets < 150×10^9/L). The presence of giant platelets in a peripheral blood film and/or reduced platelet survival time is consistent with peripheral consumption. The duration and severity of thrombocytopenia is variable and does not correlate with the course of renal disease (Kaplan and Proesmans, 1987). Bone marrow biopsy specimens usually show erythroid hyperplasia and an increased number of megakaryocytes. Prothrombin time, partial thromboplastin time, fibrinogen level, and coagulation factors are normal, thus differentiating HUS from disseminated intravascular coagulation. Mild fibrinolysis with minimal elevation in fibrin degradation products, however, may be observed. Blood leucocyte counts are usually elevated, while faecal leucocyte counts are < 10 per high-powered field in most patients, even in the presence of bloody diarrhoea (Mead and Griffin, 1998).

Evidence of renal involvement is present in all patients (by definition). Microscopic haematuria and subnephrotic proteinuria are the most consistent urinary findings.

The spectrum of the laboratory abnormalities among the patients may be very broad. Some patients develop mild elevation of LDH and serum creatinine levels, mild thrombocytopenia, and no neurological symptoms, while others develop severe acute kidney injury with oligoanuria, LDH levels > 1000 mg/dL, and neurological symptoms.

Pathology

Glomerular capillaries are the main site of damage in STEC-HUS. The predominant pathological appearances are glomerular thrombotic microangiopathy, characterized by a widened subendothelial space, endothelial cell swelling and thrombosis in capillaries and arterioles, with congested glomeruli (Inward et al., 1997). Arteriolar thrombosis is common mainly at the hilum of glomeruli and sometimes also proximally, involving interlobular arteries. Arteriolar thrombosis probably represents retrograde propagation of thrombus following stasis. Cortical infarcts are observed in cases with extensive thrombosis. Mesangial hypercellularity with basement membrane duplication and crescents may be rarely observed (Inward et al., 1997). Raised numbers of neutrophils are observed within the glomeruli, which suggest a role for inflammatory cells in the pathogenesis of glomerular lesions (Inward et al., 1997).

A report of the autopsy findings in patients with STEC-HUS revealed significant extrarenal pathology. Large bowel involvement with areas of haemorrhagic necrosis and ulceration was the commonest finding in about 45% of cases. One-third of cases had lesions in the central nervous system with intracranial haemorrhage. This included not only large subdural haematomas but also parenchymal haemorrhages ranging from frequent extensive petechiae to rare haemorrhagic infarctions (Gallo and Gianantonio, 1995). Almost 30% of patients showed lesions in the pancreas, mainly thrombotic microangiopathy in the islands of Langerhans, or in the heart with thrombotic microangiopathy in small myocardial vessels accompanied by spotty necrosis (Gallo and Gianantonio, 1995).

Aetiology and pathogenesis

After food contaminated by STEC is ingested, the toxin is released into the gut and may cause watery, or most often, bloody diarrhoea because of a direct effect on the intestinal mucosa. Stx-producing *E.coli* closely adhere to the epithelial cells of the gastrointestinal mucosa causing destruction of brush border villi (Donnenberg et al., 1993). Stxs are transported to the intracellular space of polarized gastrointestinal cells via transcellular pathways and then translocate into the circulation (Acheson et al., 1996), facilitated by the transmigration of neutrophils (polymorphonuclear neutrophils) (Hurley et al., 2001), which increase paracellular permeability. Circulating human blood cells, such as erythrocytes (Bitzan et al., 1994), platelets (Cooling et al., 1998), and monocytes (van Setten et al., 1996) express Stx receptors on their surface and have been suggested to serve as Stx carriers from the intestine to the kidney and other target organs.

The disease is caused by two distinct exotoxins—Stx-1 and Stx-2—almost identical to the toxin produced by *Shigella dysenteriae* type 1 (O'Brien et al., 1983). Both Stx-1 and Stx-2 are 70-kDa AB5 holotoxins comprising a single A subunit of 32 kDa and five 7.7-kDa B subunits. Interestingly, an AB5 toxin comprising a single 35-kDa A subunit and a pentamer of 13-kDa B subunits has been isolated from a highly virulent *E. coli* strain (O113:H21) responsible for an outbreak of HUS. This AB5 toxin may represent the prototype of a new class of toxins, accounting for HUS associated with strains of *E. coli* that do not produce Stxs (Paton et al., 2004). Despite their similar sequences, Stx-1 and Stx-2 cause different degrees and types of tissue damage as documented by the higher pathogenicity of strains of *E. coli* that produce only Stx-2 than of those that produce Stx-1 alone (Scotland et al., 1987; Ostroff et al., 1989; Cimolai et al., 1990). In a study in children who became infected by STEC, *E. coli* strains producing Stx-2 were most commonly associated with HUS, whereas most strains isolated from children with diarrhoea alone or remaining asymptomatic only produced Stx-1 (Jenkins et al., 2003). This is also true in mice and baboons (Siegler et al., 2003).

Stx-1 and Stx-2 bind to different epitopes on the Gb3 molecules and they also differ in binding affinity and kinetics (Nakajima

et al., 2001). Surface plasmon resonance analysis showed that Stx-1 easily binds to and detaches from Gb3, in contrast to Stx-2, which binds slowly but also dissociates very slowly, thus leaving sufficient time for the toxin to be incorporated into the cell (Nakajima et al., 2001).

In the kidney, Stxs bind mainly to glomerular endothelial cells but also to podocytes, mesangial cells, and proximal tubules, as shown by immunohistochemical staining (Uchida et al., 1999; Chaisri et al., 2001). These results, however, are based on a limited sample and caution is warranted before generalizing findings.

After binding to endothelial cell receptors, the toxin is internalized in the cell within 2 hours and inhibits proteins synthesis (Sandvig et al., 1989; Petruzziello-Pellegrini and Marsden, 2012). The number of high-affinity receptors is a major determinant of susceptibility of cells to Stxs. Therefore, cell viability and protein synthesis of endothelial cells of the kidney were reduced by 50% upon exposure to 1 pM Stx, unlike endothelial cells of umbilical vein that were viable up to greater than 1 nM exposure to the toxin. These findings are consistent with basal levels of Stx receptors 50 times higher in renal endothelium than in the umbilical cord endothelium (Zoja et al., 2010). During internalization, the alpha subunit of the toxin dissociates from the beta subunits, followed by retrograde transport of the alpha subunit to the Golgi apparatus (Petruzziello-Pellegrini and Marsden, 2012). Approximately 10% of the alpha subunit protein is removed in a trypsin-like process, resulting in a maximally active 27-kDa subunit enzyme. It is well established that this fragment is a direct inhibitor of protein synthesis and is responsible for the cytotoxic action of the toxin. Stxs selectively inactivate 60S ribosomal subunits by removing one nucleotide in the 28S ribosomal RNA in a nucleotide-specific manner (Zoja et al., 2010). Recent studies indicate that manganese can interfere with the retrograde movement of Stxs to the Golgi apparatus, leading to enhanced degradation of the toxins in lysosomes (Mukhopadhyay and Linstedt, 2012). Treatment of mice with manganese reduced lethality of Stxs.

For many years it was assumed that the only relevant biologic activity of Stxs was to block protein synthesis and thus destroy endothelial cells. However, treatment of endothelial cells with sublethal doses of Stxs, exerting minimal influence on protein synthesis, leads to increased mRNA levels and protein expression of chemokines, such as interleukin (IL)-8 and monocyte chemoattractant protein-1 (MCP-1) and cell adhesion molecules, a process preceded by nuclear factor kappa B (NF-κB) activation (Zoja et al., 2002). Adhesion molecules seem to play a critical role in mediating binding of inflammatory cells to endothelium. Indeed, Stx-2 treatment enhanced the number of leucocytes adhering and migrating across a monolayer of human endothelial cells (Morigi et al., 1995). Moreover, preventing IL-8 and MCP-1 overexpression by adenovirus-mediated NF-κB blocking, inhibited adhesion and transmigration of leucocytes (Zoja et al., 2002). Therefore, it can be inferred that Stxs, by altering endothelial cell adhesion properties and metabolism, favour leucocyte-dependent inflammation and induce loss of thromboresistance in endothelial cells, leading to microvascular thrombosis. Evidence for such a sequence of events has been obtained in experiments of whole blood flowing on human microvascular endothelial cells, pre-exposed to Stx-1, at high shear stress (Morigi et al., 2001). In these circumstances early platelet activation and adhesion occurs, followed by formation of organized endothelial P-selectin and platelet-endothelial cell adhesion molecule (PECAM)-1-dependent thrombi. This offers a likely pathophysiologic pathway for microvascular thrombosis in HUS.

Recent findings indicate that Stxs enhance endothelial cell expression of the chemokine receptor CXCR4 and its ligand stromal cell-derived factor-1 (SDF-1). Specific blockade of this ligand-receptor system ameliorated Stx-induced HUS in mice (Petruzziello-Pellegrini et al., 2012). Interestingly, plasma levels of SDF-1 were nearly fourfold higher in children with Stx-HUS (Petruzziello-Pellegrini et al., 2012). SDF-1 has been shown to enhance platelet activation by low levels of ADP or thrombin, leading to increased aggregation through CXCR4. Therefore, increased SDF-1 levels may be an important mechanism involved in STEC-HUS pathogenesis.

Evidence is also emerging that complement activation at renal endothelial level may contribute to microangiopathic lesions in STEC-HUS. High plasma levels of complement activation products Bb and C5b-9, were recently measured in 17 children with STEC-HUS, indicating complement activation via alternative pathway (Thurman et al., 2009). Another study reported that Stx-2 binds to the plasma complement regulatory protein, factor H, and may activate complement in the fluid phase *in vitro* (Orth et al., 2009). In a recent study, Stx-induced complement activation, via P-selectin, was identified as a key mechanism of microvascular thrombosis in STEC-HUS. Stx-1 induced the expression of P-selectin on cultured human microvascular endothelial cell surface, and P-selectin bound and activated C3 via the alternative pathway, leading to thrombus formation under flow conditions (Morigi et al., 2011). In a murine model of HUS obtained by co-injection of Stx-2 and LPS and characterized by thrombocytopenia and renal dysfunction, upregulation of glomerular endothelial P-selectin was associated with C3 and fibrin deposits and platelet clumps. Treatment with anti-P-selectin Ab limited glomerular C3 accumulation. Factor B-deficient mice after Stx-2/LPS exhibited less thrombocytopenia and were protected against glomerular abnormalities and renal function impairment, indicating the involvement of complement activation—via the alternative pathway—in the glomerular thrombotic process in HUS mice (Morigi et al., 2011).

Other renal cells express Stx receptors and are targeted by Stx. *In vitro*, tubular epithelial and mesangial cells are as susceptible to the cytotoxic effects of Stx-1 and Stx-2 as endothelial cells. In mesangial cells, Stxs inhibit protein synthesis and proliferation without altering cell viability (van Setten et al., 1997). In human proximal and distal tubular epithelial cells, specific Stx binding to Gb3 receptors has been shown, with subsequent inhibition of protein synthesis and induction of apoptosis in a dose-dependent manner (Kodama et al., 1999). The tubular damage caused by Stxs can lead to a reduction in the renal water handling capacity. Stx-2 and its B subunit inhibit water absorption across human renal tubular epithelial cell monolayers without altering short circuit current, mannitol permeability and inulin transport, indicating the integrity of the paracellular pathway (Silberstein et al., 2008). Stx administration to rats leads to a reduction in aquaporin 2 protein levels in the kidney and an elevation in aquaporin 2 excretion in the urine (Sugatani et al., 2002). These data suggest that the binding of Stx-2 B subunit to the receptor may directly affect the membrane mechanisms related to water reabsorption,

which may contribute to the early events in the pathogenesis of renal dysfunction in STEC-HUS.

Treatment and outcome

Therapy

Treatment of typical STEC-HUS in children is based on supportive management of anaemia, renal failure, hypertension, and electrolyte and water imbalance. Intravenous isotonic volume expansion as soon as an *E. coli* O157:H7 infection is suspected—that is within the first 4 days of illness, even before culture results are available—may limit the severity of kidney dysfunction and the need for renal replacement therapy (Ake et al., 2005). Up to 80% of patients receive packed red blood cells for symptomatic anaemia. Patients with severe STEC-HUS require careful monitoring, including urine output, weight, volume status, cardiovascular/respiratory function and early signs of central nervous system or other organ involvement.

Bowel rest is important for the enterohaemorrhagic colitis associated with STEC-HUS. Antimotility agents should be avoided since they may prolong the persistency of *E. coli* in the intestinal lumen and therefore increase patient exposure to its toxin. The use of antibiotics should be restricted to the very limited number of patients presenting with bacteraemia (Chiurchiu et al., 2003) since in children with gastroenteritis they may increase the risk of HUS by 17-fold (Wong et al., 2000). A possible explanation is that antibiotic-induced injury to the bacterial membrane might favour the acute release of large amounts of pre-formed toxin. Alternatively, antibiotic therapy might give *E. coli* O157:H7 a selective advantage if these organisms are not as readily eliminated from the bowel as are the normal intestinal flora. This might specifically apply to the O104:H4 strain, which has acquired a broad spectrum of resistance to antibiotics commonly used in human disease, such as cephalosporins, monobactams, fluorochinolones, co-trimoxazole, tetracyclines, and aminoglycosides which are in large part mediated by ESBL (Frank et al., 2011). Such antibiotic resistance may offer a selective advantage over the normal intestinal flora upon exposure to one or more of the above antimicrobial agents administered at the onset of gastrointestinal symptoms (Ruggenenti and Remuzzi, 2011). Moreover, several antimicrobial drugs, particularly the quinolones, trimethoprim, and furazolidone, are potent inducers of the expression of the *Stx-2* gene and may increase the level of toxin in the intestine. Although the possibility of a cause-and-effect relationship between antibiotic therapy and increased risk of HUS has been challenged by a recent meta-analysis of 26 reports (Safdar et al., 2002), there is no indication at present to prescribe antibiotics. An interesting exception could be represented by azithromycin that has been recently proposed to have some beneficial effect on duration of bacterial shedding in STEC-HUS. In a group of 65 STEC-HUS patients from the German O104:H4 epidemic, studied at a single centre in Lubeck, the 22 patients who received azithromycin had a lower frequency of long-term STEC carriage (> 28 days) than the 43 who did not receive antibiotic treatment (Nitschke et al., 2012). These findings warrant confirmation for other STEC strains, as well as prospective evaluation and clinical trials.

When haemorrhagic colitis is caused by *Shigella dysenteriae* type 1, early and empirical antibiotic treatment shortens the duration of diarrhoea, decreases the incidence of complications, and reduces the risk of transmission by shortening the duration of bacterial shedding. Thus, in developing countries where *Shigella* is the most frequent cause of haemorrhagic colitis, antibiotic therapy should be started early and even before the involved pathogen is identified.

Careful blood pressure control and renin–angiotensin system blockade may be particularly beneficial in the long term for those patients who suffer chronic renal disease after an episode of STEC-HUS. A study in 45 children with renal sequelae of HUS followed for 9–11 years documented that early restriction of proteins and use of angiotensin-converting enzyme inhibitors (ACEIs) may have a beneficial effect on long-term renal outcome, as documented by a positive slope of 1/Cr values over time in treated patients (Caletti et al., 2004). In another study, 8–15-year treatment with ACEIs after severe STEC-HUS normalized blood pressure, reduced proteinuria, and improved glomerular filtration rate (GFR) (Van Dyck and Proesmans, 2004).

An oral Stx-binding agent that may compete with endothelial and epithelial receptors for Stx in the gut (SYNSORB Pk) has been developed with the rationale of limiting target organs exposure to the toxin (Table 174.2). However, a prospective, randomized, double blind, placebo-controlled, clinical trial of 145 children with diarrhoea-associated HUS failed to demonstrate any beneficial effect of treatment on disease outcome (Trachtman et al., 2003). Among newer treatments for STEC-HUS, development of Stx-neutralizing monoclonal antibodies, including dual antibodies against Stx-1 and -2 (SHIGATEC, NCT0152199), to be given at the time of gastrointestinal infection is the most advanced (Bitzan 2009). Peptides impairing STEC ability to survive under the acidic conditions of the gastric system could halt the disease process at even earlier stages by preventing bacterial intrusion into the gut (Lino et al., 2011). Heparin and antithrombotic agents may increase the risk of bleeding and should be avoided.

Efficacy of specific treatments in adult patients is difficult to evaluate, since most information is derived by uncontrolled series that may include also atypical HUS cases. In particular, no prospective, randomized trials are available to definitely establish whether plasma infusion or exchange may offer some specific benefit as compared to supportive treatment alone. However, comparative analyses of two large series of patients treated (Dundas et al., 1999) or not (Carter et al., 1987) with plasma suggest that plasma therapy may decrease the overall mortality of STEC 0157:H7 associated HUS. Plasma infusion or exchange may therefore be considered in adult patients with severe renal insufficiency and central nervous system involvement.

Kidney transplant should be considered as an effective and safe treatment for those children who progress to end-stage renal disease (ESRD). Indeed, recurrence rates range from 0% to 10% (Artz et al., 2003; Loirat and Niaudet, 2003) and graft survival at 10 years is even better than in control children with other diseases (Ferraris et al., 2002).

Evidence that uncontrolled complement activation may contribute to microangiopathic lesions of STEC-HUS (Thurman et al., 2009; Orth and Wurzner, 2010; Morigi et al., 2011) provided the background for complement inhibitor therapy in three children with severe STEC-HUS who fully recovered with the anti-C5 monoclonal antibody eculizumab (Lapeyraque et al., 2011b). These encouraging results prompted nephrologists to use eculizumab therapy in HUS in the STEC O104:H4 outbreak in Germany. In the setting of a multicentre, single-arm, open-label

Table 174.2 Specific therapies used in HUS and TTP, dosing, and efficacy

Therapy	Dosing	Efficacy
Shiga toxin-binding (Synsorb)	500 mg/kg per day for 7 days	Not effective in preventing or treating STEC-associated HUS
Immunosuppressive:		
Prednisone	200 mg tapered to 60 mg/day then 5 mg reduction per week	Probably effective in addition to plasma exchange in patients with TTP and anti-ADAMST13 autoantibodies or in aHUS with anti-factor H autoantibodies and in forms associated with autoimmune diseases. Lack of evidence from controlled trials in immune-mediated HUS or TTP
Prednisolone	200 mg tapered to 60 mg/day then 5 mg reduction per week	
Immunoglobulins	400 mg/kg/day	
CD20 cell-depleting (rituximab)	375 mg/m^2 per week up to CD20 depletion	Effective in treatment or prevention of TTP associated with immune-mediated ADAMTS 13 deficiency resistant to, or relapsing after, immunosuppressive therapy
Fresh frozen plasma:		
Exchange	1–2 plasma volumes/day	First-line therapy for aHUS and TTP. Unproven efficacy in childhood STEC-HUS
Infusion	20–30 mL/kg followed by 10–20 mL/kg/day	Effective in prophylaxis of relapses in congenital TTP
Cryosupernatant	See plasma infusion/exchanges	To replace whole plasma in case of plasma resistance or sensitization
Solvent-detergent treated plasma	See plasma infusion/exchanges	To limit the risk of infections
Liver-kidney transplant		To prevent CFH-associated HUS recurrence post-transplant. About 30% mortality risk
Complement inhibition (eculizumab)	900 mg weekly for the first 4 weeks 1200 mg every 14 days up to 6 months	Proven efficacy in aHUS on native kidneys and in the kidney grafts

28-week clinical study (EudraCT, 2011-002691-17; Clinicaltrials.gov ID: NCT01410916) 328 patients with STEC-HUS, received eculizumab treatment. Preliminary results have been reported on 148 treated patients by Dr Rolf Stahl from the Hamburg University Medical Center during the 43th Annual Meeting of the American Society of Nephrology held in Denver in November 2011. At the onset, 94 patients were on dialysis, 22 required ventilator support, and 129 were receiving plasma therapy. At 8 weeks of eculizumab treatment, platelet count and serum creatinine concentration normalized in 123 and 82 patients, respectively, and no patient died or had persistent seizures. However, other authors reported no significant difference in treatment efficacy between patients of the same German epidemic who received eculizumab together with plasma exchange (N = 189) and those who received plasma exchange alone (N = 229) (Kielstein, 2012). Whether eculizumab is a useful adjunct to treating the most severe forms of STEC-HUS need to be clarified by prospective randomized, controlled trials.

Clinical course

More than 90% of childhood cases of STEC-HUS fully recover from acute disease. In a report from the Walkerton Health Study on 19 children with STEC-HUS due to an *E.coli* O157:H7 outbreak, none required long-term dialysis and all had a serum creatinine that returned to the normal range (Garg et al., 2009). However STEC-HUS can have serious long-term consequences for kidney function. A meta-analysis of 49 published studies (3476 patients, mean follow-up of 4.4 years) describing the long-term prognosis of patients who survived an episode of STEC-HUS, reported death or permanent ESRD in 12% of patients and a GFR < 80 mL/min/1.73 m^2 in 25% (Garg et al., 2003). The severity of acute illness, particularly central nervous system symptoms, the need for initial dialysis, and microalbuminuria in the first 6–8 months were strongly associated with a worse long-term prognosis.

Neuraminidase-associated haemolytic uraemic syndrome

Aetiology

This is a rare (Table 174.1) but potentially fatal disease that may complicate pneumonia, or less frequently, meningitis caused by *Streptococcus pneumoniae* (Brandt et al., 2002). Neuraminidase produced by *Streptococcus pneumoniae*, cleaves *N*-acetylneuraminic acid from the glycoproteins on the cell membrane of erythrocytes, platelets, and glomerular cells (Cochran et al., 2004). Removing the *N*-acetylneuraminic acid exposes the normally hidden Thomsen–Friedenreich antigen (T-antigen) (McGraw et al., 1989), which can then react with anti-T IgM antibody naturally present in human plasma. This antigen–antibody reaction occurs more frequently in infants and children and causes polyagglutination of red blood cells *in vitro*. This is the reason why, unlike in other forms of HUS, in neuraminidase-associated HUS there is a positive Coombs test. T-anti-T interaction on red cells, platelets, and endothelium was thought to explain the pathogenesis, whereas the pathogenic role of the anti-T cold antibody *in vivo* is uncertain (Eder and Manno, 2001). T-antigen exposure on red cells is detected using the lectin *Hypogeae*.

Clinical course and therapy

Patients, usually < 2 years old, present with severe microangiopathic haemolytic anaemia. The clinical picture is severe, with respiratory distress, neurological involvement, and coma. The acute mortality is about 25%.

The outcome is strongly dependent on the effectiveness of antibiotic therapy. In theory, plasma either infused or exchanged, is contraindicated, since adult plasma contains antibodies against the Thomsen–Friedenreich antigen that may accelerate polyagglutination and haemolysis (McGraw et al., 1989). Thus, patients should be treated only with antibiotics and washed red cells. In some cases, however, plasma therapy, occasionally in combination with steroids, has been associated with recovery.

Atypical haemolytic uraemic syndrome

Epidemiology

Atypical haemolytic uraemic syndrome (aHUS) (Table 174.1) is significantly less common than STEC-HUS, accounting for approximately 5% of all types of HUS (Noris and Remuzzi, 2009). The incidence in the United States has been reported to be approximately 2 per million population per year (Constantinescu et al., 2004) but European data suggest that it may be lower than this at about 0.5 per million population per year. aHUS affects all ages with a peak incidence under the age of 18 (Noris et al., 2010). Both sexes are equally affected in childhood (Sellier-Leclerc et al., 2007). In adults, the incidence and prevalence is greater in females (Sullivan et al., 2010).

Both sporadic and familial forms of aHUS are described (Kavanagh and Goodship, 2010). Various precipitants including non-enteric infections, drugs, malignancies, transplantation, and pregnancy have been described in the pathogenesis of aHUS. In approximately 20% of female patients with aHUS, the disease is associated with pregnancy (George, 2003; Fakhouri et al., 2010; Goodship and Kavanagh, 2010); the disease tends to occur at term or postpartum. Pregnancy-associated increased concentrations of pro-coagulant factors, decreased fibrinolytic activity, and reduced expression of endothelial thrombomodulin may be predisposing factors. Drugs associated with aHUS include cytotoxics, immunosuppressants, and antiplatelet agents (Dlott et al., 2004). Other factors that have been recognized to precede the onset of the disease include diarrhoea, respiratory infection, and malignant hypertension (Noris et al., 2010). Determining whether preceding diarrhoea is secondary to a STEC infection is important as specific treatment for aHUS becomes available.

Inherited and acquired factors associated with aHUS

aHUS can be both familial and sporadic (Noris and Remuzzi, 2009). That aHUS could be familial has been recognized for many years (Kaplan et al., 1975; Berns et al., 1992; Kaplan and Leonard 2000). The inheritance was initially thought to be predominantly recessive (Kaplan et al., 1997) but it is now recognized that non-penetrance is a common feature which confounds the interpretation of inheritance; most families are dominant with impaired penetrance. In 1998, Warwicker et al. published the results of a linkage study in three families with aHUS (Warwicker et al., 1998). This showed segregation of the disease to an area on chromosome 1q32 containing a cluster of genes important in the regulation of complement activation (the RCA cluster). That complement abnormalities were associated with aHUS had been recognized for many years. In particular, both low levels of C3 and glomerular deposition of C3 had been reported (Stuhlinger et al., 1974; Hammar et al., 1978; Carreras et al., 1981; Noris et al., 1999). The first candidate gene to be screened in the RCA cluster was factor H (*CFH*) because previous reports had shown an association between factor H deficiency and aHUS (Thompson and Winterborn, 1981; Pichette et al., 1994; Ohali et al., 1998). *CFH* mutation screening of the three families showed that in one of the families all affected members carried a heterozygous missense mutation in the C-terminal exon. Since then a series of studies have established in both the familial and sporadic forms of aHUS that dysregulation and/or excessive activation of the alternative pathway of complement plays a pivotal role in the pathogenesis of the disease (Fig. 174.1). In both forms, inherited and/or acquired abnormalities affecting components of the alternative complement pathway are found in approximately 60% of patients. These include mutations in the genes encoding both complement regulators (factor H, factor I, membrane cofactor protein, and thrombomodulin) and activators (factor B and C3); and autoantibodies against factor H and factor I. Approximately 20% of patients carry a mutation in more than one of these genes (Table 174.1).

Factor H

Factor H and other proteins within the RCA cluster share a common basic structure consisting of multiple (contiguous) homologous modules called short complement regulator (SCR) domains or complement control protein (CCP) modules. Factor H has 20 SCRs each comprising approximately 60 amino acids. Mutations in *CFH* in aHUS patients have been widely described (Caprioli et al., 2001, 2006; Perez-Caballero et al., 2001; Richards et al., 2001; Manuelian et al., 2003; Dragon-Durey et al., 2004), are listed at the interactive FH-HUS mutation database (<http://www.FH-HUS.org>) (Saunders et al., 2006, 2007), and are found in approximately 30% of patients. The majority are heterozygous missense mutations that cluster in the C-terminal exons and are associated with normal factor H levels. A minority are deletions or missense mutations that result in either a truncated protein or impaired secretion. This leads to systemic factor H deficiency, usually heterozygous.

The clustering of missense mutations in the C-terminal region of the molecule is remarkable. The alternative pathway is activated by 'foreign' surfaces. It exhibits spontaneous activity which is regulated by factor H. Factor H acts as a cofactor for factor I-mediated cleavage of C3b (cofactor activity) and accelerates the decay of the C3 convertase C3bBb (decay accelerating activity) (Fig. 174.1). It also competes with factor B for binding to C3b and binds to polyanions on cell surfaces. The C-terminal region of factor H where mutations cluster is known to be important in the latter two functions. Structural models suggest that it is impairment of the ability to inactivate surface bound C3b in particular that is important in the pathogenesis of aHUS (Kajander et al., 2011; Morgan et al., 2011). Functional studies have confirmed this finding (Sanchez-Corral et al., 2002; Manuelian et al., 2003; Sanchez-Corral et al., 2004; Jozsi et al., 2006; Lehtinen et al., 2009). To further test this *in vivo*, mouse models of aHUS-associated *CFH* mutations have been generated. A mouse completely deficient in factor H ($Cfh^{-/-}$) was found to have uncontrolled activity of the alternative pathway with very low C3 levels and a renal pathology similar to membranoproliferative

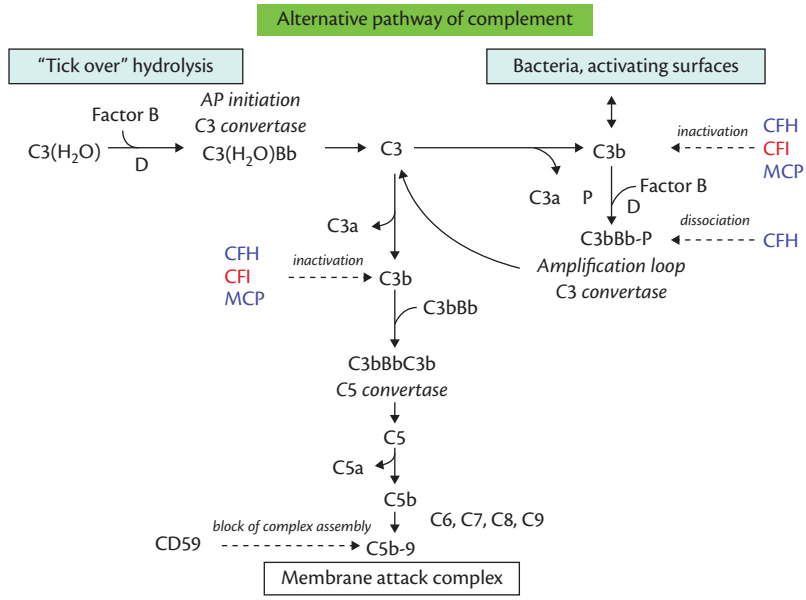

Fig. 174.1 The alternative pathway of complement activation. The alternative pathway is continuously activated in plasma by low-grade hydrolysis of C3 that binds factor B, to form a C3(H_2O)B complex. Factor D cleaves factor B to form the alternative pathway initiation C3 convertase that cleaves C3 to C3b that binds to target cells such as bacterial cells and together with factor B form the amplification loop C3 convertase C3bBb. The binding of properdin stabilizes this enzyme. C3 convertase enzymes cleave many molecules of C3 to form the anaphylatoxins C3a, and C3b that forms additional C3 convertase, resulting in a positive feedback amplification loop. C3b also binds to the C3 convertase forming the C5 convertase enzyme $C3b_2Bb$ that cleaves C5 to the anaphylatoxin C5a and C5b. The latter then initiates the formation of the membrane-attack complex. The human complement system is highly regulated through a number of membrane-anchored and fluid phase regulators that inactivate complement products formed at various levels in the cascade and protect host tissues.
CD59 = protectin (prevents the polymerization of the membrane attack complex); CFB = complement factor B; CFH = complement factor H (acts as a cofactor for factor I for C3b cleavage and favours the decay of the C3 convertase of the alternative pathway); CFI = complement factor I (in red colour, degrades C3b and C4b); MCP = membrane cofactor protein (binds C3b and C4b and has cofactor activity for both the classical and the alternative pathways).

glomerulonephritis (MPGN), not aHUS (Pickering et al., 2002). In contrast a mouse lacking the C-terminal end of factor H (*Cfh*$^{-/-}$Δ16–20), designed to mimic the mutations commonly seen in aHUS had higher plasma C3 levels than the *Cfh*$^{-/-}$ mouse, and spontaneously develops aHUS, not MPGN (Pickering et al., 2007). Missense mutations are also found to a lesser extent in the N-terminal region of the molecule where they have been shown to impair the regulatory function of factor H (Pechtl et al., 2011).

Genomic disorders affecting *CFH* have also been described in association with aHUS. Complement genes within the RCA cluster at chromosome 1q32 are arranged in tandem within two groups. In a centromeric 360 kb segment lie the genes for factor H (*CFH*) and five factor H-related proteins—*CFHR1, CFHR2, CFHR3, CFHR4*, and *CFHR5*. Sequence analysis of this region shows evidence of several large genomic duplications, also known as low copy repeats (LCRs), resulting in a high degree of sequence identity between *CFH* and the genes for the five factor H-related proteins (Jozsi and Zipfel, 2008). LCRs such as those seen in the RCA cluster are frequently associated with genomic rearrangements (Lupski, 2007). These usually result from either gene conversion or non-allelic homologous recombination (NAHR) between LCRs. It has been observed that some of the mutations in the C-terminal exons of *CFH* have arisen by gene conversion (Heinen et al., 2006). It has been shown that NAHR in this region can lead to the formation of a hybrid gene consisting of the first 21 exons of *CFH* (encoding SCRs 1–18 of the hybrid gene) and the last two exons of *CFHR1* (encoding SCRs 19 and 20 of the hybrid gene) (Venables et al., 2006). This hybrid gene encodes a protein product that is identical to a functionally significant factor H mutant (Ser1191Leu/Val1197Ala) which arises by gene conversion. In addition to macrohomology in the RCA cluster there is also inevitably microhomology which predisposes to deletions through microhomology-mediated end joining rather than NAHR. This has been shown to result in the formation of a *CFH/CFHR3* hybrid gene. The protein product of this is a 24-SCR protein which is secreted with normal fluid phase activity but marked loss of complement regulation at cell surfaces despite increased heparin binding (Francis et al., 2012).

Besides inherited defects in *CFH*, acquired abnormalities affecting factor H function are also seen in the form of inhibitory autoantibodies (Jozsi et al., 2007, 2008; Skerka et al., 2009; Dragon-Durey et al., 2010; Moore et al., 2010). Factor H autoantibodies are reported in 5–10% of aHUS patients. Analogous to the genetic defects seen in factor H, these autoantibodies also mainly target the C-terminal end of the protein, thereby impairing complement regulation on host cell surfaces. The development of factor-H autoantibodies in aHUS has a genetic predisposition, being strongly associated with a deletion of *CFHR1* and *CFHR3* which also arises through NAHR (Moore et al., 2010). Further analysis subsequently suggested that the association between the *CFHR1/CFHR3* deletion and the presence of autoantibodies in aHUS is probably related to the absence of factor H-related protein 1 (Abarrategui-Garrido et al., 2009; Moore et al., 2010). However, deficiency of factor H-related protein 1 is not a prerequisite for formation of autoantibodies as patients with no evidence of deficiency of either of these factor H related proteins and high titres of autoantibodies have been described (Moore et al., 2010).

Factor H and factor H-related protein 1 share a high degree of homology with the two C-terminal SCRs (4 and 5) of CFHR1 being almost identical to SCRs 19–20 of factor H. It is not surprising, therefore, that autoantibodies to SCRs 19–20 of factor H also bind to SCRs 4–5 of factor H-related protein 1 (Moore et al., 2010).

Membrane cofactor protein (CD46)

Membrane cofactor protein (MCP, CD46) is a transmembrane complement regulator widely expressed on the surface of all cells apart from erythrocytes (Goodship et al., 2004) (Fig. 174.1). The extracellular domain comprises four SCRs followed by an alternatively spliced region rich in threonine, serine, and proline (the STP region) and a group of 12 amino acids. This is followed by the transmembrane domain and an alternatively spliced cytoplasmic tail which mediates signalling events. Together with factor I, MCP degrades C3b and C4b bound to the cell surface. Two simultaneous reports (Noris et al., 2003; Richards et al., 2003) initially reported mutations in *MCP* in association with aHUS. Since then further disease-associated *MCP* mutations have been described in approximately 15% of aHUS patients (Esparza-Gordillo et al., 2005; Caprioli et al., 2006; Fremeaux-Bacchi et al., 2006; Maga et al., 2010; Noris et al., 2010). The functional effect of many of these mutations has been modelled. The majority result in reduced cell surface expression with a minority showing reduced C3b binding and cofactor activity (Richards et al., 2007).

Factor I

Factor I is a soluble serine protease of the complement system which cleaves three peptide bonds in the alpha chain of C3b and two bonds in the alpha chain of C4b thereby inactivating these proteins (Fig. 174.1). Factor H acts as a cofactor for C3b, C4 binding protein for C4b and membrane cofactor protein for both. Factor I is a heterodimer of approximately 88 kDa which consists of a non-catalytic heavy chain of 50 kDa which is linked to a catalytic light chain of 38 kDa by a disulphide bond. The protein is synthesized as a single-chain precursor of 565 amino acids, mainly in the liver. Four basic amino acids are then excised from the precursor before secretion of the heterodimer. Like many of the complement proteins, factor I has a modular structure. The heavy chain contains two low-density lipoprotein receptor (LDLr) domains, a CD5 domain, and a module found only in factor I and complement proteins C6 and C7. The gene encoding factor I is located on chromosome 4q25 and spans 63 kb. It comprises 13 exons and there is a strong correlation between the exonic organization of the gene and the modular structure of the protein. The light chain of factor I, which is the serine proteinase region of the molecule, is encoded by five exons. The genomic organization of the enzymic part of factor I is similar to that of trypsin. *CFI* mutations are found in approximately 12% of aHUS patients (Kavanagh et al., 2008; Kavanagh and Goodship, 2010). Most mutations result in heterozygous quantitative deficiency of factor I, although some mutants are secreted but have impaired proteolytic activity (Kavanagh et al., 2008; Bienaime et al., 2010). The functional effects of mutations in the heavy chain remain to be elucidated.

Factor B

Factor B carries the serine protease domain necessary for amplification of the alternative pathway (Fig. 174.1). The alternative pathway convertase is assembled in two steps. First, C3b combines with factor B to form C3bB which is then cleaved by factor D producing the fragments Ba and Bb. Ba dissociates from the complex, Bb remains bound to C3b to form the active C3 convertase C3bBb. Dissociation of the two components of this complex results in inactivation of the convertase and is promoted by decay accelerating factor, complement receptor 1 and factor H. The gene encoding factor B (*CFB*) is located on chromosome 6p21.3 and consists of 18 exons coding for a five domain glycoprotein. The N-terminal Ba fragment contains three complement control protein domains. The C-terminal Bb fragment contains two domains. The N-terminal type A domain contains the C3b-binding region and the C-terminal domain is the site of the serine protease. The frequency of *CFB* mutations in the cohorts of aHUS patients examined to date is low at < 3% (Goicoechea de Jorge et al., 2007; Roumenina et al., 2009; Maga et al., 2010; Noris et al., 2010). Functional analysis of the mutants has demonstrated either increased generation or enhanced stability of the alternative pathway convertase.

C3

C3 is the pivotal component of the complement system (Janssen and Gros, 2007). Activation of the classical, lectin, and alternative pathways results in cleavage of C3 to generate C3b and the anaphylatoxin C3a. When C3b is produced, the thioester is cleaved and this highly reactive species C bind covalently to targets. Interaction of factor B with C3b and subsequent cleavage of factor B by factor D results in formation of the alternative pathway C3 convertase C3bBb (Fig. 174.1). This represents an amplification loop. A series of complement regulators including factor H and membrane cofactor protein prevent amplification through this loop by increasing the rate of dissociation of C3bBb and/or by serving as cofactors for factor I. The gene encoding C3 is located on chromosome 19 and comprises 41 exons, 16 of which encode the β chain and 25 the α chain. Mutations in C3 have been described in approximately 10% of aHUS (Fremeaux-Bacchi et al., 2008; Maga et al., 2010; Noris et al., 2010). Functional analysis of five of the nine mutations revealed increased resistance to complement regulation and in another two, the mutations resulted in a decreased secretion of C3 (Fremeaux-Bacchi et al., 2008). The mechanism by which impaired C3 secretion results in aHUS is as yet unclear.

Thrombomodulin

Thrombomodulin is a key component of the protein C anticoagulation pathway where it facilitates the activation of protein C by thrombin. Additionally it enhances thrombin-mediated activation of plasma procarboxypeptidase B (TAFI) an inhibitor of fibrinolysis that also inactivates complement-derived anaphylatoxins C3a and C5a. Thrombomodulin has been shown to downregulate the alternative pathway of complement by accelerating factor I mediated inactivation of C3b in the presence of co-factors. Mutations in the gene encoding thrombomodulin have recently been shown to predispose to atypical HUS (Delvaeye et al., 2009; Maga et al., 2010; Noris et al., 2010). These mutations resulted in a loss of cofactor activity.

Factors affecting the development of aHUS

Characteristic of the familial form of aHUS is that approximately 50% of individuals will not manifest aHUS despite carrying a mutation in one of the aforementioned genes. Two other factors are thought to determine the development of the disease (Fig. 174.2). First, in most patients there is a trigger. Infection and pregnancy

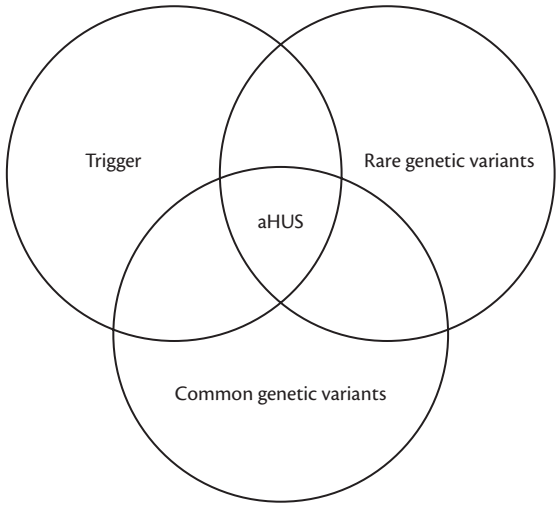

Fig. 174.2 Manifestation of aHUS in an individual may need the presence of a trigger such as pregnancy, a rare genetic variant such as a mutation in a complement gene, and a common genetic variant such as an at-risk haplotype in a complement gene.
Adapted from Kavanagh and Goodship (2010). Reproduced with permission of American Society of Hematology (ASH).

are the most frequently described triggers (Fakhouri et al., 2010; Noris et al., 2010). Second, a further genetic variant (modifier) can increase the risk of developing the disease. This can be in the form of either an additional mutation in one of the aforementioned genes and/or the presence of a common at-risk genetic variant. It is now recognized that approximately 20% of aHUS patients will have mutations in more than one gene (Maga et al., 2010; Noris et al., 2010). Common at-risk genetic variants (single nucleotide polymorphisms (SNPs) and haplotype blocks) in *CFH, CD46*, and *CFHR1* have been shown to act as susceptibility factors for the development of the disease (Martinez-Barricarte et al., 2008; Abarrategui-Garrido et al., 2009). Thus the presence of a rare genetic variant (mutation), a common at-risk genetic variant (SNPs and haplotype blocks), and a trigger may be necessary for the disease to be manifest (Rodriguez de Cordoba, 2010).

Investigation and treatment of aHUS

The increased understanding of the molecular mechanisms responsible for the development of aHUS have underpinned the development of national and international guidelines for the investigation and treatment of the disease (Ariceta et al., 2009; Saland et al., 2009a; Taylor et al., 2010). Box 174.1 shows the investigations recommended in patients presenting with the clinical features of aHUS.

Currently plasma exchange and/or plasma infusions is the recommended first-line management (Table 174.2). Plasma exchange is commonly undertaken daily using 1–2 plasma volumes per session in adults and 50–100 mL/kg in children. Typically plasma exchange is undertaken daily initially, the duration and frequency of treatment is then determined by the clinical response. Patients with abnormalities in soluble regulators such as factor H respond better to plasma exchange than patients with abnormalities in the transmembrane regulator MCP (Noris et al., 2010). Despite treatment with plasma therapy the majority of patients progress to end-stage renal failure within 3 years of presentation (Noris et al., 2010). While no problems have been reported with the use of haemodialysis and peritoneal dialysis in aHUS the use of transplantation has been controversial. The risk of recurrent HUS post transplant is approximately 50% but is significantly greater (~ 80%) in patients known to have a mutation in either *CFH* or *CFI* (Bresin et al., 2006). In contrast, the outcome for patients known to have only an *MCP* mutation is favourable (Kavanagh et al., 2010). Because both factor H and factor I are produced mainly by the liver, combined liver/kidney transplantation is a logical therapeutic option. Whilst the outcome in initial reports of this modality was less favourable (Remuzzi et al., 2002a), more recent reports (Saland et al., 2006; Jalanko et al., 2008; Saland et al., 2009b; Wilson et al., 2011) suggest that a favourable long-term outcome is possible. Pivotal to this is the use of prophylactic plasma exchange immediately prior to surgery (Saland et al., 2009a). Another option is to undertake a renal transplant alone with prophylactic plasma exchange preoperatively, postoperatively, and long term. While this approach has been used successfully (Davin et al., 2008), recurrent disease can occur and patients may become intolerant of plasma exchange.

Box 174.1 Investigations recommended in a patient presenting with the clinical features of aHUS

- ADAMTS13 activity to exclude TTP
- Evidence of gastrointestinal *Escherichia coli* infection to exclude STEC-associated HUS
- C3, C4, factor H, and factor I levels
- MCP (CD46) expression on PBMCs
- Mutation screening of CFH, CFI, CD46, CFB, C3, and THBD
- Identification of genomic disorders—deletions and hybrid genes
- Factor H autoantibodies.

For some time there has been enthusiasm for the potential use of complement inhibitors in aHUS. Recent anecdotal case reports of the use of the anti-C5 monoclonal antibody eculizumab in aHUS have been encouraging (Table 174.2). In total there have been 13 published reports (Chatelet et al., 2009; Gruppo and Rother, 2009; Mache et al., 2009; Nurnberger et al., 2009; Davin et al., 2010; Larrea et al., 2010; Zimmerhackl et al., 2010; Al-Akash et al., 2011; Lapeyraque et al., 2011a; Nester et al., 2011; Duran et al. 2012; Garjau et al. 2012; Kim et al. 2012). The patients' ages ranged from 18 months to 32 years. In six of these patients, eculizumab was used to treat recurrent disease post renal transplantation (four showed complete recovery and two partial). In six, eculizumab was given for aHUS affecting their native kidneys (two showed complete recovery and four partial recovery). In one patient the eculizumab was given prophylactically to cover a renal transplant in a patient with a factor H abnormality. The successful outcome in these anecdotal reports led to multicentre trials of eculizumab being undertaken in aHUS. Based on these results, a licence for the use of eculizumab in aHUS has been granted by both the US Food and Drug Administration and the European Medicines Agency.

Thrombotic thrombocytopenic purpura

Epidemiology and classification

TTP (Table 174.1) is a rare disease, with an incidence of approximately 2–4 per million population/year (Crowther and George,

2008; Galbusera et al., 2006). It is more common in women (female:male ratio, 3:2–5:2) and in white people (white:black ratio, 3:1). Although the peak incidence is in the third and fourth decades of life, TTP can affect any age group (Galbusera et al., 2006; George, 2006).

TTP may be primary and results from autoantibodies that inhibit the activity of ADAMTS13, a plasma metalloprotease that cleaves von Willebrand factor (vWF), or rarely from *ADAMTS13* gene defects (Galbusera et al., 2006).

Secondary TTP may occur in relation to cancer, haematopoietic stem cell or solid organ transplantation, chemotherapy, pregnancy, and certain drugs among which anti-aggregating agents such as ticlopidine and clopidogrel and calcineurin inhibitors such as ciclosporin and tacrolimus are best known (Table 174.1). The mechanism leading to the development of a TTP episode in secondary TTP mostly remains unclear; ADAMTS13 deficiency is rare (Galbusera et al., 2006).

Clinical features and investigations

TTP classically presents with the pentad of thrombocytopenia, microangiopathic haemolytic anaemia, fever, and neurological and renal dysfunction (Moschcowitz, 2003). Thrombocytopenia is essential for the diagnosis, most patients present with values < 60,000/μL (Galbusera et al., 2006; George, 2006). Most, but not all patients have very high levels of LDH (George, 2010). Anaemia usually becomes more severe during the week after diagnosis (George, 2010).

Purpura is minor and can be absent. Retinal haemorrhages can be present; however, bleeding is rare. Neurological symptoms can be seen in > 90% of patients during the entire course of the disease. Central nervous system involvement mainly represents thrombo-occlusive disease of the grey matter, but can also include headache, cranial nerve palsies, confusion, stupor, and coma. These features are transient but recurrent. Up to half of patients who present with neurological involvement may be left with sequelae. Renal insufficiency may occur. One group has reported 25% of patients to have creatinine clearance < 40 mL/min (Eknoyan and Riggs, 1986). Microscopic haematuria and subnephrotic proteinuria are the most consistent urinary abnormalities. In a retrospective study of 216 patients with a clinical picture of TTP, haematuria was detected in 78% and proteinuria in 75% of cases (Eknoyan and Riggs, 1986). Sterile pyuria and casts were present in 31% and 24% of cases, respectively. Gross haematuria was rare. Low-grade fever is present in one-quarter of patients at diagnosis, but can often be seen as a consequence of plasma exchange. Other common presenting features are gastrointestinal symptoms, possibly related to intestinal ischaemia, and symptoms of weakness and fatigue, possibly related to anaemia. Cardiac involvement may be common in TTP (George, 2010). In one study, some patients had elevations of serum troponin I, but evidence for acute myocardial infarction was rare (George, 2010). Less common manifestations include pancreatitis and sudden death (George 2006, 2010).

The typical pathologic changes of TTP are widespread hyaline thrombi, accompanied by variable fibroblastic infiltration and endothelial overlay, in the terminal arterioles and capillaries of multiple organs. The thrombi are found most extensively in the heart, brain, kidney, pancreas, spleen, mesentery, and adrenal gland, and are composed primarily of platelets and vWF (Tsai, 2010). Fibrin may be present surrounding or sometimes penetrating the amorphous or granular materials. In older lesions, hyaline deposits may be seen in the subendothelial layers of capillaries and between the endothelium and muscular layers of arterioles. Pre-occlusive pseudoaneurysmal dilatation may also be present. Fibrinoid necrosis and vascular or perivascular inflammatory cell infiltration are characteristically absent or minimally (Tsai, 2010).

Compared to HUS, pathologic changes of TTP are more extensively distributed, probably reflecting the more systemic nature of the disease (Remuzzi et al., 1994).

Aetiology and pathogenesis

In the microvasculature of patients with TTP, systemic platelet thrombi develop, mainly formed by platelets and von Willebrand Factor (vWF). This protein plays a major role in primary haemostasis forming platelet plugs at sites of vascular injury under high shear stress. vWF is a large glycoprotein synthesized in vascular endothelial cells and megakaryocytes as a high-molecular-weight polymer (Moake, 1998). Secretion of vWF occurs primarily via the Weibel–Palade bodies. Exocytosis of vWF from the storage granules may be swiftly increased by exposing endothelial cells to secretagogues such as histamine, thrombin, phorbol myristate acetate or calcium ionophores. Upon stimulation, vWF is secreted by endothelial cells as ultra-large (UL) multimers that form string-like structures attached to the endothelial cells, possibly through interaction with P-selectin (Padilla et al., 2004). Under fluid shear stress, the UL-vWF strings are cleaved to generate the range of vWF multimer sizes that normally circulate in the blood, from approximately 500 kDa to 20 million Da (Sadler 2008). The proteolytic cleavage of vWF multimers appears to be critical to prevent thrombosis in the microvasculature. ADAMTS13 is the protease responsible for cleaving vWF at the peptide bond Y1605-M1606 in the A2 domain, creating 140- and 176-kD fragments (Fig. 174.3).

ADAMTS13 is encoded by the homonymous gene located at chromosome 9q34 (Levy et al., 2001). It is a zinc metalloprotease consisting of 1427 amino acid residues and has a nodular structure comprising a N-terminal signal peptide, a propeptide, a reprolysin-like metalloprotease domain, a disintegrin-like domain, a first thrombospondin type-1 motif (TSP1), a cysteine-rich domain, a spacer domain, seven additional TSP1 repeats, and two CUB (C1r/C1s, urinary epidermal growth factor, bone morphogenic protein) domains. Plasma ADAMTS13 derives primarily from the stellate cells of the liver (Zhou et al., 2005). ADAMTS13 may also be expressed, albeit at lower levels, in other types of cells such as renal podocytes and tubular cells (Tsai 2010), vascular endothelial cells (Turner et al., 2006) and platelets (Tsai, 2010). The plasma concentration of ADAMTS13 is approximately 1 microgram/mL, or 5 nmol/L. The eliminated half-life of ADAMTS13 is 1–2 days in the circulation.

The interaction between ADAMTS13 and vWF is complex. The catalytic site of ADAMTS13 resides in the metalloprotease domain. The sequence downstream of the metalloprotease domain also interacts with vWF, thereby facilitating the attack on the vWF scissile bond by the ADAMTS13 catalytic site. Similarly, shear stress makes vWF susceptible to cleavage by ADAMTS13 by unfolding vWF multimers to an elongated form and exposing the scissile bonds (Tsai et al., 1994).

ADAMTS13 is deficient in the majority of patients with primary TTP leading to accumulation of UL-vWF multimers that are highly reactive with platelets (Furlan et al., 1998a; Tsai and Lian 1998). Exposure of UL-vWF multimers and platelets to shear stress leads

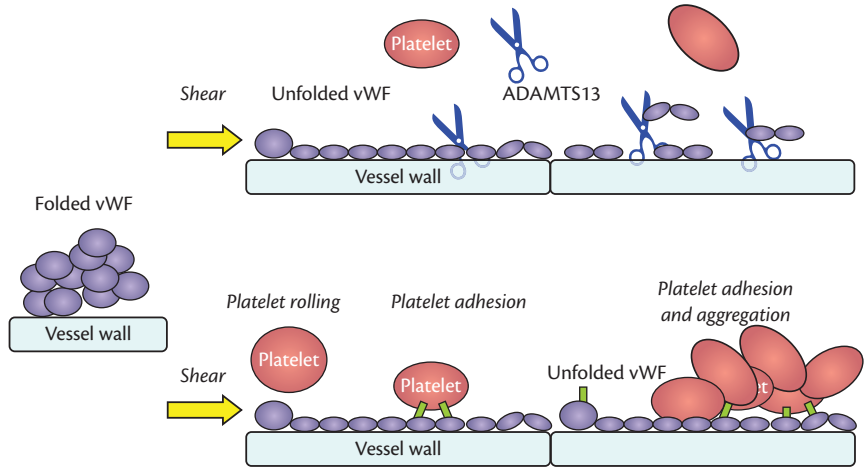

Fig. 174.3 The pathophysiology of platelet thrombosis in TTP. Upon stimulation, vWF ultra-large (UL) multimers are secreted by endothelial cells into the circulation in a folded structure. Upon exposure to enhanced shear stress UL multimers form string-like structures that adhere to endothelial cells. Normally, UL-vWF strings are cleaved by ADAMTS13 to generate vWF multimers from 500 kDa to 20 million Da molecular weight to prevent thrombosis in the microvasculature (upper panel). When the ADAMTS13 proteolytic activity is defective because of the inhibitory effect of anti-ADAMTS13 autoantibodies or *ADAMTS13* gene mutations, UL-vWF multimers accumulate and interact with activated platelets to facilitate platelet adhesion and aggregation, with thrombi formation and occlusion of the vascular lumen (lower panel).

to platelet aggregation (Donadelli et al., 2006a). The levels of shear stress necessary for inducing platelet aggregation is in the range found in normal arteriolar and capillary circulation. Therefore vWF and platelets have a propensity to form aggregates in normal arterioles and capillaries that needs to be constantly regulated. By cleaving UL-vWF multimers before they are activated by shear stress to cause platelet aggregation, ADAMTS13 prevents spontaneous microvascular thrombosis in the normal circulation.

The platelet aggregation observed in patients with TTP and ADAMTS13 deficiency is thus a direct consequence of the appearance of UL-vWF multimers in the patient's circulation. Consequently, microvascular thrombi occur in almost all organ vessels, resulting in diffuse organ ischaemia and thrombocytopenia secondary to platelet consumption. Microangiopathic haemolysis is caused by the passage of blood through the damaged capillaries and arterioles occluded by thrombi (Moake, 2002).

Two mechanisms for deficiency of the ADAMTS13 activity have been identified in patients with primary TTP, an acquired deficiency due to the formation of anti-ADAMTS13 autoantibodies (acquired TTP), and a genetic deficiency due to homozygous or compound heterozygous mutations in the *ADAMTS13* gene (congenital TTP) (Table 174.1).

TTP associated with immune-mediated deficiency of ADAMTS13

This is an immune-mediated, acquired form of TTP that accounts for the majority of cases (60–90%) reported to date as acute primary or sporadic TTP. The disease is characterized by a severe deficiency of ADAMTS13 (Tsai and Lian, 1998) whose activity is inhibited by specific autoantibodies that develop transiently and tend to disappear during remission (Table 174.1) (Tsai and Lian, 1998; Veyradier et al., 2001; Ferrari et al., 2007). These inhibitory anti-ADAMTS13 antibodies are mainly immunoglobulin (Ig)-G (Furlan et al., 1998b; Tsai and Lian, 1998; Ferrari et al., 2007), although IgM and IgA anti-ADAMTS13 antibodies have also been described (Ferrari et al., 2007).

Patients with TTP secondary to haematopoietic stem cell transplantation, malignancies or HIV infection rarely have severe ADAMTS13 deficiency and inhibitory IgG antibodies (Vesely et al., 2003; Zheng et al., 2004; Zheng and Sadler, 2008). TTP associated with ticlopidine and clopidogrel (thienopyridine drugs that inhibit platelet aggregation) represent interesting exceptions of secondary TTP consistent with a drug-induced autoimmune disorder. Severe ADAMTS13 deficiency and ADAMTS13 inhibitory antibodies were detected in 80–90% of patients with ticlopidine-associated TTP (Tsai et al., 2000; Zheng and Sadler, 2008) and in a few patients with clopidogrel-induced TTP (Zheng and Sadler, 2008). The deficiency resolved after the drugs were discontinued. ADAMTS13 inhibitors have not been described in other drug-associated TTP (quinine, mitomycin C and other cancer chemotherapeutic agents, calcineurin inhibitors) (Park et al., 2009). TTP diagnosed during pregnancy comprises approximately 7% of all TTP cases (George et al., 2008). The majority of these cases have acquired ADAMTS13 deficiency (Gerth et al., 2009), but pregnancy has also been reported as a triggering event in patients with genetic ADAMTS13 deficiency (see below).

The ADAMTS13 epitopes recognized by autoantibodies have been mapped and so far all positive serum samples contain at least some antibodies directed against the Cys-rich/spacer domain (Soejima et al., 2003; Klaus et al., 2004). In some cases, the antibodies were directed only against this epitope, but in the majority of patients combinations of antibodies were found, including antibodies against CUB domains, the TSP1 repeats, and the ADAMTS13 propeptide (Klaus et al., 2004). When cloned and prepared as monoclonal antibodies, many of these antibodies inhibit ADAMTS13 activity *in vitro*, and *in vivo* in mice (Zheng and Sadler, 2008).

Evidence for the pathogenicity of TTP-associated anti-ADAMTS13 autoantibodies is derived from the observation that they usually disappear from the circulation when remission is achieved by effective treatment and this occurs in parallel with the normalization of ADAMTS13 activity. In patients with acquired ADAMTS13 deficiency, a risk as high as 50% to develop relapses

has been reported, and undetectable ADAMTS13 activity and persistence of anti-ADAMTS13 inhibitors during remission predict recurrences (Ferrari et al., 2007).

TTP associated with congenital deficiency of ADAMTS13

This rare inherited form is associated with a genetic defect of ADAMTS13 and accounts for about 5% of all of cases of TTP (Table 174.1) (Galbusera et al., 2009). Emerging data indicate that patients with a clinical phenotype of HUS (Veyradier et al., 2001; Remuzzi et al., 2002b) may have a complete lack of ADAMTS13 activity, albeit less frequently. Thus on clinical ground a possible inherited defect of ADAMTS13 cannot be excluded on the basis of predominance of renal involvement. TTP associated with congenital ADAMTS13 deficiency can present both with and without a family history of the disease (Furlan et al., 1998a; Levy et al., 2001; Veyradier et al., 2001). In both cases the disease is inherited as a recessive trait, as documented by the fact that ADAMTS13 levels in unaffected relatives of patients fall into a bimodal distribution with a group with half normal levels, consistent with carriers, and the other with normal values.

To date, > 100 ADAMTS13 mutations has been identified in patients with TTP (Levy et al., 2001; Donadelli et al., 2006b; Galbusera et al., 2009; Loirat et al., 2013) (<http://www.ttpdatabase.org>). Approximately 60% of these mutations are missense, causing single amino acid substitutions, and the remaining are nonsense, deletions or insertions causing frameshifts, or splice site mutants leading to a truncated protein. Only a few mutations have been described in more than one pedigree. A notable exception is 4143insA, which has been described in multiple pedigrees of Northern and Central Europe and in Turkey. Haplotype analysis suggested that most of the 4143insA mutant alleles probably originated from a common ancestry (Schneppenheim et al., 2006).

Studies on secretion and activity of the mutated forms of ADAMTS13 showed that most of these mutations led to impaired secretion of the protease from the cells, and when the mutated protein is secreted the proteolytic activity is greatly reduced (Galbusera et al., 2009).

Most patients are carriers of compound heterozygous mutations; only 20% of mutations have been observed in homozygous form. Heterozygous individuals have ADAMTS13 activity in the 40–70% range of normal values and do not have any phenotypic abnormality (Tsai, 2010).

Treatment and outcome

Therapy

Plasma therapy is the cornerstone of therapy in an acute episode. Plasma may serve to induce remission of the disease by replacing defective protease activity. In patients with anti-ADAMTS13 antibodies, as compared to infusion, exchange may offer the advantage of also rapidly removing anti-ADAMTS13 antibodies (Table 174.2). (George, 2010)

Because of the potential for sudden clinical deterioration, treatment should be initiated as soon as possible after diagnosis. The treatment approach consists of a daily one to two plasma volume exchange with plasma until clinical symptoms have resolved and the platelet count has reached a normal level (≥ 150,000/μL). Fresh frozen plasma (FFP), 24-hour plasma, and cryosupernatant plasma are considered equivalent because of comparable levels of ADAMTS13 (Scott et al., 2007).

After beginning plasma exchange, the next decision is whether to begin corticosteroid therapy (Table 174.2). Corticosteroids may be of benefit in autoimmune forms of TTP by inhibiting the synthesis of anti-ADAMTS13 autoantibodies. In a series of 33 patients with undetectable ADAMTS13 activity and anti-ADAMTS13 antibodies, combined treatment with plasma exchange and prednisone was associated with disease remission in around 90% of cases (Ferrari et al., 2007). The rationale of combined treatment is that plasma exchange will have only a temporary effect on the presumed autoimmune basis of the disease and additional immunosuppressive treatment may cause a more durable response. Thirty out of 108 patients with either TTP or HUS were reported to have recovered after treatment with corticosteroids alone. All of them, however, had mild forms and none of them were tested for ADAMTS13 activity (Ruggenenti et al., 2008).

Recent prospective studies have successfully and safely used rituximab (Table 174.2), an anti-CD20 monoclonal antibody depleting B lymphocytes, in patients who had failed to respond to standard daily plasma exchange and methylprednisolone and in patients with relapsed acute TTP who had previously demonstrated antibodies to ADAMTS13 (Fakhouri et al., 2005; Scully et al., 2007). Treatment was associated with clinical remission in all patients, disappearance of anti-ADAMTS13 antibodies, and with increase of ADAMTS13 activity to levels > 10%.

A study conducted by the French Thrombotic Microangiopathies Reference Center enrolled 22 adults with TTP who had either refractory disease or disease exacerbation following standard plasma exchange therapy. Patients were given additional rituximab therapy—four infusions (375 mg/m^2) over 7 days (Ireland, 2012). One patient with refractory disease died after two infusions of rituximab; the other 21 patients all received four rituximab infusions as first-line salvage therapy and achieved prolonged remission. Time to prolonged remission was shorter in rituximab-treated patients than in 53 historical controls who had received plasma exchange alone and survived. After 35 days, recovery of platelet count had occurred in all 21 rituximab-treated patients but only 78% of historical controls. No relapses were recorded in the rituximab-treated patients, whereas about 10% of historical controls relapsed during this time (Ireland, 2012). However long-term relapse rates (> 1 year) did not differ between groups (Ireland, 2012). Of the about 100 rituximab-treated patients reported in the literature so far, normalization of platelets and LDH has been noted in about 95%, however time to remission has been variable, from 1 to 4 weeks after the first dose. The duration of remission has ranged between 9 months and 4 years, with relapses reported in approximately 10% (Kiss, 2010).

Rituximab has been also used electively to prevent relapses in patients with autoantibodies and recurrent disease (Fakhouri et al., 2005; Galbusera et al., 2005; Bresin et al., 2009). In a study, five patients with persistent undetectable ADAMTS13 activity and high titres of autoantibody were treated with rituximab as pre-emptive therapy during remission. ADAMTS13 activity ranged from 15% to 75% and absence of autoantibodies was seen after 3 months in all patients, and activity was still > 20% at 6 months. Three patients maintained a disease-free status after 29, 24, and 6 months, respectively (Bresin et al., 2009). Relapses were documented at 13 and 51 months in the remaining two patients during follow-up. Longitudinal evaluation of ADAMTS13 activity and autoantibodies levels may help monitoring patient response

to treatment. Re-treatment with rituximab should be considered when ADAMTS13 activity decreases and inhibitors reappear into the circulation, to prevent a relapse.

In patients suffering from TTP associated with congenital ADAMTS13 deficiency, ADAMTS13 is constitutively lacking and can be replaced by plasma infusions (Barbot et al., 2001; Kremer Hovinga and Meyer, 2008). During acute episodes of TTP, patients often require plasma exchange to restore a stable clinical and laboratory state. Providing sufficient ADAMTS13 to achieve 5% normal enzymatic activity may be sufficient to degrade large vWF multimers—which may be relevant to induce remission of the microangiopathic process—and this effect is sustained over time due to the relatively long half-life of the protease. Infused ADAMTS13 has a plasma half-life of 2–3 days *in vivo* (Furlan et al., 1999), and although plasma levels fall below 5% within 3–7 after fresh FFP administration, the effect on platelet count and clinical parameters may last up to 3 weeks, suggesting that ADAMTS13 remains available, for example, on platelets and endothelial cells. In two brothers with complete deficiency of the protease and relapsing TTP, disease remission was achieved by plasma exchange and was concurrent with an almost full recovery of the ADAMTS13 activity. Both patients achieved a long-lasting remission, although protease activity decreased to < 10% over 20 days after plasma therapy withdrawal (Furlan et al., 1999).

After a first episode of TTP has occurred, patients with congenital ADAMTS13 deficiency tend to relapse. Patients with frequent relapses, a severe clinical course with neurological sequelae, renal insufficiency, and patients who have siblings who have died of TTP, should be put on regular prophylactic FFP infusions. These patients should receive 10–20 mL FFP/kg body weight per session every 2–3 weeks, a regimen that has been shown to be effective in preventing acute TTP bouts and maintaining the patients in good health for years (Barbot et al., 2001; Kremer Hovinga and Meyer, 2008).

Clinical course

Compared to patients with non-severe ADAMTS13 deficiency, severely deficient patients experience higher rate of remissions (82–88% vs 20–75%) and lower mortality (8–18% vs 18–80%) (Mori et al., 2002; Vesely et al., 2003; Zheng et al., 2004). The high mortality rate in non-severely deficient patients may be due to the higher proportion of secondary causes and death from underlying diseases, such as patients with haematological malignancies (Kiss, 2010).

Among patients who have a severe ADAMTS13 deficiency, patients with inhibitory antibodies experience a more severe manifestation of the disease, take substantially longer time to achieve clinical remission, and require a higher plasma volume than patients with congenital ADAMTS13 deficiency (Coppo et al., 2006). Neurological symptoms usually dominate the clinical picture and may be fleeting and fluctuating, probably because of continuous thrombi formation and dispersion in the brain microcirculation. Coma and seizures complicate the most severe forms. The detection of high titres of anti-ADAMTS13 autoantibodies is correlated with relapsing disease and poor prognosis. Also the persistence of inhibitory antibodies at clinical remission is predictive of relapse rate: a study found a threefold higher risk of relapse in patients with continuing ADAMTS13 deficiency and inhibitor present compared to those without inhibitors at remission (Peyvandi et al., 2008).

TTP has been reported in 1 every 1600–5000 patients treated with ticlopidine. Eleven cases have been reported during treatment with clopidogrel, a new antiaggregating agent that has achieved widespread clinical use for its safety profile. Most patients with TTP associated with ticlopidine or clopidogrel had neurological involvement. The overall survival rate is 67% and is improved by early treatment withdrawal and plasma therapy.

Approximately 60% of patients with congenital deficiency of ADAMTS13 experience their first acute episode of disease in the neonatal period or during infancy, but a second group (10–20%) manifests the disease after the third decade of life. TTP recurrences are common but their frequency varies widely. While some patients with congenital ADAMTS13 deficiency depend on frequent chronic plasma infusions to prevent recurrences, many patients who achieved clinical remission after plasma treatment remain in a disease-free status for long periods of time after plasma discontinuation, despite the absence of protease activity (Galbusera et al., 2009).

Emerging data suggest that the type and location of ADAMTS13 mutations may influence the age of onset of TTP and the penetrance of the disease in mutation carriers (Galbusera et al., 2009). One of the most frequently reported ADAMTS13 mutation, the 4143-4144insA in the second CUB domain, leading to a frameshift and loss of the last 49 amino acids of the protein, is associated with neonatal-childhood onset, indeed only one out of 16 reported carriers, either homozygous or compound heterozygous with other ADAMTS13 mutations, reached the adult age without developing TTP (Schneppenheim et al., 2006; Galbusera et al., 2009). *In vitro* expression studies revealed that the mutation causes a severe impairment of protein secretion combined with a strongly reduced specific protease activity. On the other hand, mutations in the sixth and the seventh TSP1 (Galbusera et al., 2009; Palla et al., 2009) appear to lead to an adult onset and a milder course of TTP. Expression studies revealed that these mutations result in severe defects in secretion of the metalloprotease, although a small fraction of the mutant protein is released in the supernatant, but the mutants maintain normal specific protease activity (Tao et al., 2006; Galbusera et al., 2009). It is possible that in carriers of these mutations small ADAMTS13 activity may be present in the circulation, which is enough to prevent onset of the disease in childhood or even in adulthood. The latter possibility is supported by descriptions of asymptomatic carriers of such mutations who never developed TTP (Donadelli et al., 2006b; Galbusera et al., 2009; Palla et al., 2009).

Recently, a correlation between ADAMTS13 mutations and age of TTP onset has been documented, reflecting indirectly the severity of patients' phenotypes (Lotta et al., 2012). In the same study, patients carrying mutations associated with some residual ADAMTS13 activity in blood had a longer disease-free survival compared to patients with completely absent protease activity (Lotta et al., 2012).

Environmental factors may contribute to induce full-blown manifestation of the disease. According to this 'two-hit model', deficiency of ADAMTS13 predisposes to microvascular thrombosis and thrombotic microangiopathy supervenes after a triggering event that activates microvascular endothelial cells and causes the secretion of UL-vWF multimers and P-selectin expression. Potential triggers of the above phenomena are infections and pregnancy. Six women with congenital ADAMTS13 deficiency

developed late-onset TTP during pregnancy (Donadelli et al., 2006b; Camilleri et al., 2008). Also, genetic modifiers may be implicated in susceptibility to develop thrombotic microangiopathy in conditions of ADAMTS13 deficiency, which may include genes encoding proteins involved in the regulation of the coagulation cascade, vWF, or platelet function, components of the endothelial vessel surface or of the complement cascade.

References

Abarrategui-Garrido, C., Martinez-Barricarte, R., Lopez-Trascasa, M., *et al.* (2009). Characterization of complement factor H-related (CFHR) proteins in plasma reveals novel genetic variations of CFHR1 associated with atypical hemolytic uremic syndrome. *Blood*, 114, 4261–71.

Acheson, D. W., Moore, R., De Breucker, S., *et al.* (1996). Translocation of Shiga toxin across polarized intestinal cells in tissue culture. *Infect Immun*, 64, 3294–300.

Akashi, S., Joh, K., Tsuji, A., *et al.* (1994). A severe outbreak of haemorrhagic colitis and haemolytic uraemic syndrome associated with Escherichia coli O157:H7 in Japan. *Eur J Pediatr*, 153, 650–5.

Ake, J. A., Jelacic, S., Ciol, M. A., *et al.* (2005). Relative nephroprotection during Escherichia coli O157:H7 infections: association with intravenous volume expansion. *Pediatrics*, 115, e673–80.

Al-Akash, S. I., Almond, P. S., Savell, V. H., Jr., *et al.* (2011). Eculizumab induces long-term remission in recurrent post-transplant HUS associated with C3 gene mutation. *Pediatr Nephrol*, 26, 613–19.

Ariceta, G., Besbas, N., Johnson, S., *et al.* (2009). Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. *Pediatr Nephrol*, 24, 687–96.

Artz, M. A., Steenbergen, E. J., Hoitsma, A. J., *et al.* (2003). Renal transplantation in patients with hemolytic uremic syndrome: high rate of recurrence and increased incidence of acute rejections. *Transplantation*, 76, 821–6.

Banatvala, N., Griffin, P. M., Greene, K. D., *et al.* (2001). The United States National Prospective Hemolytic Uremic Syndrome Study: microbiologic, serologic, clinical, and epidemiologic findings. *J Infect Dis*, 183, 1063–70.

Barbot, J., Costa, E., Guerra, M., *et al.* (2001). Ten years of prophylactic treatment with fresh-frozen plasma in a child with chronic relapsing thrombotic thrombocytopenic purpura as a result of a congenital deficiency of von Willebrand factor-cleaving protease. *Br J Haematol*, 113, 649–51.

Beatty, M. E., Griffin, P. M., Tulu, A. N., *et al.* (2004). Culturing practices and antibiotic use in children with diarrhea. *Pediatrics*, 113, 628–9.

Belongia, E. A., Osterholm, M. T., Soler, J. T., *et al.* (1993). Transmission of Escherichia coli O157:H7 infection in Minnesota child day-care facilities. *JAMA*, 269, 883–8.

Berns, J. S., Kaplan, B. S., Mackow, R. C., *et al.* (1992). Inherited hemolytic uremic syndrome in adults. *Am J Kidney Dis*, 19, 331–4.

Besbas, N., Karpman, D., Landau, D., *et al.* (2006). A classification of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders. *Kidney Int*, 70, 423–31.

Bienaime, F., Dragon-Durey, M. A., Regnier, C. H., *et al.* (2010). Mutations in components of complement influence the outcome of Factor I-associated atypical hemolytic uremic syndrome. *Kidney Int*, 77, 339–49.

Bitzan, M. (2009). Treatment options for HUS secondary to Escherichia coli O157:H7. *Kidney Int Suppl*, S62–6.

Bitzan, M., Richardson, S., Huang, C., *et al.* (1994). Evidence that verotoxins (Shiga-like toxins) from Escherichia coli bind to P blood group antigens of human erythrocytes in vitro. *Infect Immun*, 62, 3337–47.

Blaser, M. J. (2011). Deconstructing a lethal foodborne epidemic. *N Engl J Med*, 365, 1835–6.

Brandt, J., Wong, C., Mihm, S., *et al.* (2002). Invasive pneumococcal disease and hemolytic uremic syndrome. *Pediatrics*, 110, 371–6.

Bresin, E., Daina, E., Noris, M., *et al.* (2006). Outcome of renal transplantation in patients with non-Shiga Toxin-associated haemolytic uremic syndrome: prognostic significance of genetic background. *Clin J Am Soc Nephrol*, 1, 88–99.

Bresin, E., Gastoldi, S., Daina, E., *et al.* (2009). Rituximab as pre-emptive treatment in patients with thrombotic thrombocytopenic purpura and evidence of anti-ADAMTS13 autoantibodies. *Thromb Haemost*, 101, 233–8.

Buchholz, U., Bernard, H., Werber, D., *et al.* (2011). German outbreak of Escherichia coli O104:H4 associated with sprouts. *N Engl J Med*, 365, 1763–70.

Caletti, M. G., Lejarraga, H., Kelmansky, D., *et al.* (2004). Two different therapeutic regimes in patients with sequelae of hemolytic-uremic syndrome. *Pediatr Nephrol*, 19, 1148–52.

Camilleri, R. S., Cohen, H., Mackie, I. J., *et al.* (2008). Prevalence of the ADAMTS-13 missense mutation R1060W in late onset adult thrombotic thrombocytopenic purpura. *J Thromb Haemost*, 6, 331–8.

Caprioli, J., Bettinaglio, P., Zipfel, P. F., *et al.* (2001). The molecular basis of familial hemolytic uremic syndrome: mutation analysis of factor H gene reveals a hot spot in short consensus repeat 20. *J Am Soc Nephrol*, 12, 297–307.

Caprioli, A., Morabito, S., Brugere, H., *et al.* (2005). Enterohaemorrhagic Escherichia coli: emerging issues on virulence and modes of transmission. *Vet Res*, 36, 289–311.

Caprioli, J., Noris, M., Brioschi, S., *et al.* (2006). Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. *Blood*, 108, 1267–79.

Carreras, L., Romero, R., Requesens, C., *et al.* (1981). Familial hypocomplementemic hemolytic uremic syndrome with HLA-A3,B7 haplotype. *JAMA*, 245, 602–4.

Carter, A. O., Borczyk, A. A., Carson, J. A., *et al.* (1987). A severe outbreak of Escherichia coli O157:H7-associated hemorrhagic colitis in a nursing home. *N Engl J Med*, 317, 1496–500.

Centers for Disease Control and Prevention (1997). Outbreaks of Escherichia coli O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider—Connecticut and New York, October 1996. *MMWR Morb Mortal Wkly Rep*, 46, 4–8.

Chaisri, U., Nagata, M., Kurazono, H., *et al.* (2001). Localization of Shiga toxins of enterohaemorrhagic Escherichia coli in kidneys of paediatric and geriatric patients with fatal haemolytic uraemic syndrome. *Microb Pathog*, 31, 59–67.

Chapman, P. A., Siddons, C. A., Gerdan Malo, A. T., *et al.* (1997). A 1-year study of Escherichia coli O157 in cattle, sheep, pigs and poultry. *Epidemiol Infect*, 119, 245–50.

Chatelet, V., Fremeaux-Bacchi, V., Lobbedez, T., *et al.* (2009). Safety and long-term efficacy of eculizumab in a renal transplant patient with recurrent atypical hemolytic-uremic syndrome. *Am J Transplant*, 9, 2644–5.

Chiurchiu, C., Firrincieli, A., Santostefano, M., *et al.* (2003). Adult nondiarrhea hemolytic uremic syndrome associated with Shiga toxin Escherichia coli O157:H7 bacteremia and urinary tract infection. *Am J Kidney Dis*, 41, E4.

Cimolai, N., Carter, J. E., Morrison, B. J., *et al.* (1990). Risk factors for the progression of Escherichia coli O157:H7 enteritis to hemolytic-uremic syndrome. *J Pediatr*, 116, 589–92.

Cochran, J. B., Panzarino, V. M., Maes, L. Y., *et al.* (2004). Pneumococcus-induced T-antigen activation in hemolytic uremic syndrome and anemia. *Pediatr Nephrol*, 19, 317–21.

Constantinescu, A. R., Bitzan, M., Weiss, L. S., *et al.* (2004). Non-enteropathic hemolytic uremic syndrome: causes and short-term course. *Am J Kidney Dis*, 43, 976–82.

Cooling, L. L., Walker, K. E., Gille, T., *et al.* (1998). Shiga toxin binds human platelets via globotriaosylceramide (Pk antigen) and a novel platelet glycosphingolipid. *Infect Immun*, 66, 4355–66.

Coppo, P., Wolf, M., Veyradier, A., *et al.* (2006). Prognostic value of inhibitory anti-ADAMTS13 antibodies in adult-acquired thrombotic thrombocytopenic purpura. *Br J Haematol*, 132, 66–74.

Crowther, M. A. and George, J. N. (2008). Thrombotic thrombocytopenic purpura: 2008 update. *Cleve Clin J Med*, 75, 369–75.

Davin, J. C., Gracchi, V., Bouts, A., *et al*. (2010). Maintenance of kidney function following treatment with eculizumab and discontinuation of plasma exchange after a third kidney transplant for atypical hemolytic uremic syndrome associated With a CFH mutation. *Am J Kidney Dis*, 55, 708–11.

Davin, J. C., Strain, L., and Goodship, T. H. (2008). Plasma therapy in atypical haemolytic uremic syndrome: lessons from a family with a factor H mutation. *Pediatr Nephrol*, 23, 1517–21.

Delvaeye, M., Noris, M., DeVriese, A., *et al*. (2009). Mutations in thrombomodulin in hemolytic-uremic syndrome. *N Engl J Med*, 361, 345–57.

Dlott, J. S., Danielson, C. F., Blue-Hnidy, D. E., *et al*. (2004). Drug-induced thrombotic thrombocytopenic purpura/hemolytic uremic syndrome: a concise review. *Therap Apher Dial*, 8, 102–11.

Donadelli, R., Banterla, F., Galbusera, M., *et al*. (2006b). In-vitro and in-vivo consequences of mutations in the von Willebrand factor cleaving protease ADAMTS13 in thrombotic thrombocytopenic purpura. *Thromb Haemost*, 96, 454–64.

Donadelli, R., Orje, J.N., Capoferri, C., *et al*. (2006a). Size regulation of von Willebrand factor-mediated platelet thrombi by ADAMTS13 in flowing blood. *Blood*, 107, 1943–50.

Donnenberg, M. S., Tacket, C. O., James, S. P., *et al*. (1993). Role of the eaeA gene in experimental enteropathogenic Escherichia coli infection. *J Clin Invest*, 92, 1412–7.

Dragon-Durey, M. A., Fremeaux-Bacchi, V., Loirat, C., *et al*. (2004). Heterozygous and homozygous factor h deficiencies associated with hemolytic uremic syndrome or membranoproliferative glomerulonephritis: report and genetic analysis of 16 cases. *J Am Soc Nephrol*, 15, 787–95.

Dragon-Durey, M. A., Sethi, S. K., Bagga, A., *et al*. (2010). Clinical features of anti-factor H autoantibody-associated hemolytic uremic syndrome. *J Am Soc Nephrol*, 21, 2180–7.

Dundas, S., Murphy, J., Soutar, R. L., *et al*. (1999). Effectiveness of therapeutic plasma exchange in the 1996 Lanarkshire Escherichia coli O157:H7 outbreak. *Lancet*, 354, 1327–30.

Duran, C. E., Blasco, M., Maduell, F., *et al*. (2012). Rescue therapy with eculizumab in a transplant recipient with atypical heamolytic-uraemic syndrome. *Clin Kidney J*, 5, 28–30.

Eder, A. F. and Manno, C. S. (2001). Does red-cell T activation matter? *Br J Haematol*, 114, 25–30.

Eknoyan, G. and Riggs, S. A. (1986). Renal involvement in patients with thrombotic thrombocytopenic purpura. *Am J Nephrol*, 6, 117–31.

Esparza-Gordillo, J., Goicoechea de Jorge, E., Buil, A., *et al*. (2005). Predisposition to atypical hemolytic uremic syndrome involves the concurrence of different susceptibility alleles in the regulators of complement activation gene cluster in 1q32. *Hum Mol Genet*, 14, 703–12.

Fakhouri, F., Roumenina, L., Provot, F., *et al*. (2010). Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations. *J Am Soc Nephrol*, 21, 859–67.

Fakhouri, F., Vernant, J. P., Veyradier, A., *et al*. (2005). Efficiency of curative and prophylactic treatment with rituximab in ADAMTS13-deficient thrombotic thrombocytopenic purpura: a study of 11 cases. *Blood*, 106, 1932–7.

Ferrari, S., Scheiflinger, F., Rieger, M., *et al*. (2007). Prognostic value of anti-ADAMTS 13 antibody features (Ig isotype, titer, and inhibitory effect) in a cohort of 35 adult French patients undergoing a first episode of thrombotic microangiopathy with undetectable ADAMTS 13 activity. *Blood*, 109, 2815–22.

Ferraris, J. R., Ramirez, J. A., Ruiz, S., *et al*. (2002). Shiga toxin-associated hemolytic uremic syndrome: absence of recurrence after renal transplantation. *Pediatr Nephrol*, 17, 809–14.

Francis, N. J., McNicholas, B., Awan, A., *et al*. (2012). A novel hybrid CFH/CFHR3 gene generated by a microhomology-mediated deletion in familial atypical hemolytic uremic syndrome. *Blood*, 119, 591–601.

Frank, C., Werber, D., Cramer, J. P., *et al*. (2011). Epidemic profile of Shiga-toxin-producing Escherichia coli O104:H4 outbreak in Germany. *N Engl J Med*, 365, 1771–80.

Fremeaux-Bacchi, V., Miller, E. C., Liszewski, M. K., *et al*. (2008). Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. *Blood*, 112, 4948–52.

Fremeaux-Bacchi, V., Moulton, E. A., Kavanagh, D., *et al*. (2006). Genetic and functional analyses of membrane cofactor protein (CD46) mutations in atypical hemolytic uremic syndrome. *J Am Soc Nephrol*, 17, 2017–25.

Furlan, M., Robles, R., Galbusera, M., *et al*. (1998a). von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *N Engl J Med*, 339, 1578–84.

Furlan, M., Robles, R., Morselli, B., *et al*. (1999). Recovery and half-life of von Willebrand factor-cleaving protease after plasma therapy in patients with thrombotic thrombocytopenic purpura. *Thromb Haemost*, 81, 8–13.

Furlan, M., Robles, R., Solenthaler, M., *et al*. (1998b). Acquired deficiency of von Willebrand factor-cleaving protease in a patient with thrombotic thrombocytopenic purpura. *Blood*, 91, 2839–46.

Galbusera, M., Bresin, E., Noris, M., *et al*. (2005). Rituximab prevents recurrence of thrombotic thrombocytopenic purpura: a case report. *Blood*, 106, 925–8.

Galbusera, M., Noris, M. and Remuzzi, G. (2006). Thrombotic thrombocytopenic purpura—then and now. *Semin Thromb Hemost*, 32, 81–9.

Galbusera, M., Noris, M. and Remuzzi, G. (2009). Inherited thrombotic thrombocytopenic purpura. *Haematologica*, 94, 166–70.

Gallo, E. G. and Gianantonio, C. A. (1995). Extrarenal involvement in diarrhoea-associated haemolytic-uraemic syndrome. *Pediatr Nephrol*, 9, 117–19.

Garg, A. X., Clark, W. F., Salvadori, M., *et al*. (2006). Absence of renal sequelae after childhood Escherichia coli O157:H7 gastroenteritis. *Kidney Int*, 70, 807–12.

Garg, A. X., Salvadori, M., Moist, L. M., *e al*. (2009). Renal prognosis of toxigenic Escherichia coli infection. *Kidney Int Suppl*, S38–41.

Garg, A. X., Suri, R. S., Barrowman, N., *et al*. (2003). Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: a systematic review, meta-analysis, and meta-regression. *JAMA*, 290, 1360–70.

Garjau, M., Azancot, M., Ramos, R., *et al*. (2012). Early treatment with eculizumab in atypical haemolytic uraemic syndrome. *Clin Kidney J*, 5, 31–3.

Gasser, C., Gautier, E., Steck, A., *et al*. (1955). [Hemolytic-uremic syndrome: bilateral necrosis of the renal cortex in acute acquired hemolytic anemia.]. *Schweiz Med Wochenschr*, 85, 905–9.

George, J. N. (2003). The association of pregnancy with thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. *Curr Opin Hematol*, 10, 339–44.

George, J. N. (2006). Clinical practice. Thrombotic thrombocytopenic purpura. *N Engl J Med*, 354, 1927–35.

George, J. N. (2010). How I treat patients with thrombotic thrombocytopenic purpura: 2010. *Blood*, 116, 4060–9.

George, J. N., Terrell, D. R., Swisher, K. K., *et al*. (2008). Lessons learned from the Oklahoma thrombotic thrombocytopenic purpura-hemolytic uremic syndrome registry. *J Clin Apher*, 23, 129–37.

Gerth, J., Schleussner, E., Kentouche, K., *et al*. (2009). Pregnancy-associated thrombotic thrombocytopenic purpura. *Thromb Haemost*, 101, 248–51.

Goicoechea de Jorge, E., Harris, C. L., Esparza-Gordillo, J., *et al*. (2007). Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. *Proc Natl Acad Sci U S A*, 104, 240–5.

Goodship, T. H. and Kavanagh, D. (2010). Pulling the trigger in atypical hemolytic uremic syndrome: the role of pregnancy. *J Am Soc Nephrol*, 21, 731–2.

Goodship, T. H., Liszewski, M. K., Kemp, E. J., *et al*. (2004). Mutations in CD46, a complement regulatory protein, predispose to atypical HUS. *Trends Mol Med*, 10, 226–31.

Gruppo, R. A. and Rother, R. P. (2009). Eculizumab for congenital atypical hemolytic-uremic syndrome. *N Engl J Med*, 360, 544–6.

Hammar, S. P., Bloomer, H. A., and McCloskey, D. (1978). Adult hemolytic uremic syndrome with renal arteriolar deposition of IgM andC3. *Am J Clin Pathol*, 70, 434–9.

Heinen, S., Sanchez-Corral, P., Jackson, M. S., *et al.* (2006). De novo gene conversion in the RCA gene cluster (1q32) causes mutations in complement factor H associated with atypical hemolytic uremic syndrome. *Hum Mutat*, 27, 292–3.

Hurley, B. P., Thorpe, C. M. and Acheson, D. W. (2001). Shiga toxin translocation across intestinal epithelial cells is enhanced by neutrophil transmigration. *Infect Immun*, 69, 6148–55.

Inward, C. D., Howie, A .J., Fitzpatrick, M. M., *et al.* (1997). Renal histopathology in fatal cases of diarrhoea-associated haemolytic uraemic syndrome. British Association for Paediatric Nephrology. *Pediatr Nephrol*, 11, 556–9.

Ireland, R. (2012). Thrombotic microangiopathy: rituximab in severe autoimmune TTP. *Nat Rev Nephrol*, 8, 131.

Jalanko, H., Peltonen, S., Koskinen, A., *et al.* (2008). Successful liver-kidney transplantation in two children with aHUS caused by a mutation in complement factor H. *Am J Transplant*, 8, 216–21.

Janssen, B. J. and Gros, P. (2007). Structural insights into the central complement component C3. *Mol Immunol*, 44, 3–10.

Jenkins, C., Willshaw, G. A., Evans, J., *et al.* (2003). Subtyping of virulence genes in verocytotoxin-producing Escherichia coli (VTEC) other than serogroup O157 associated with disease in the United Kingdom. *J Med Microbiol*, 52, 941–7.

Jozsi, M., Heinen, S., Hartmann, A., *et al.* (2006). Factor H and atypical hemolytic uremic syndrome: mutations in the C-terminus cause structural changes and defective recognition functions. *J Am Soc Nephrol*, 17, 170–7.

Jozsi, M., Licht, C., Strobel, S., *et al.* (2008). Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. *Blood*, 111, 1512–14.

Jozsi, M., Strobel, S., Dahse, H. M., *et al.* (2007). Anti factor H autoantibodies block C-terminal recognition function of factor H in hemolytic uremic syndrome. *Blood*, 110, 1516–18.

Jozsi, M. and Zipfel, P. F. (2008). Factor H family proteins and human diseases. *Trends Immunol*, 29, 380–7.

Kajander, T., Lehtinen, M. J., Hyvarinen, S., *et al.* (2011). Dual interaction of factor H with C3d and glycosaminoglycans in host-nonhost discrimination by complement. *Proc Natl Acad Sci U S A*, 108, 2897–902.

Kaplan, B. S. and Proesmans, W. (1987). The hemolytic uremic syndrome of childhood and its variants. *Semin Hematol*, 24, 148–60.

Kaplan, B. S., Chesney, R. W., and Drummond, K. N. (1975). Hemolytic uremic syndrome in families. *N Engl J Med*, 292, 1090–3.

Kaplan, B. S. and Leonard, M. B. (2000). Autosomal dominant hemolytic uremic syndrome: variable phenotypes and transplant results. *Pediatr Nephrol*, 14, 464–8.

Kaplan, B. S., Papadimitriou, M., Brezin, J. H., *et al.* (1997). Renal transplantation in adults with autosomal recessive inheritance of hemolytic uremic syndrome. *Am J Kidney Dis*, 30, 760–5.

Kavanagh, D. and Goodship, T. H. (2010). Atypical hemolytic uremic syndrome. *Curr Opin Hematol*, 17, 432–8.

Kavanagh, D., Richards, A., Goodship, T., *et al.* (2010). Transplantation in atypical hemolytic uremic syndrome. *Semin Thromb Hemost*, 36, 653–9.

Kavanagh, D., Richards, A., Noris, M., *et al.* (2008). Characterization of mutations in complement factor I (CFI) associated with hemolytic uremic syndrome. *Mol Immunol*, 45, 95–105.

Keene, W. E., McAnulty, J. M., Hoesly, F. C., *et al.* (1994). A swimming-associated outbreak of hemorrhagic colitis caused by Escherichia coli O157:H7 and Shigella sonnei. *N Engl J Med*, 331, 579–84.

Kielstein, J. (2012). Best supportive care and therapeutic plasma exchange. *Nephrol Dial Transplant*, 27, 373–74.

Kim, J. J., Waller, S. C., and Reid, C. J. (2012). Eculizumab in atypical haemolytic uraemic syndrome allows cessation of plasma exchange and dialysis. *Clin Kidney J*, 5, 34–6.

Kiss, J. E. (2010). Thrombotic thrombocytopenic purpura: recognition and management. *Int J Hematol*, 91, 36–45.

Klaus, C., Plaimauer, B., Studt, J. D., *et al.* (2004). Epitope mapping of ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura. *Blood*, 103, 4514–19.

Kodama, T., Nagayama, K., Yamada, K., *et al.* (1999). Induction of apoptosis in human renal proximal tubular epithelial cells by Escherichia coli verocytotoxin 1 in vitro. *Med Microbiol Immunol*, 188, 73–8.

Kremer Hovinga, J. A. and Meyer, S. C. (2008). Current management of thrombotic thrombocytopenic purpura. *Curr Opin Hematol*, 15, 445–50.

Lapeyraque, A. L., Fremeaux-Bacchi, V., and Robitaille, P. (2011a). Efficacy of eculizumab in a patient with factor-H-associated atypical hemolytic uremic syndrome. *Pediatr Nephrol*, 26, 621–4.

Lapeyraque, A. L., Malina, M., Fremeaux-Bacchi, V., *et al.* (2011b). Eculizumab in severe Shiga-toxin-associated HUS. *N Engl J Med*, 364, 2561–3.

Larrea, C. F., Cofan, F., Oppenheimer, F., *et al.* (2010). Efficacy of eculizumab in the treatment of recurrent atypical hemolytic-uremic syndrome after renal transplantation. *Transplantation*, 89, 903–4.

Lehtinen, M. J., Rops, A. L., Isenman, D. E., *et al.* (2009). Mutations of factor H impair regulation of surface-bound C3b by three mechanisms in atypical hemolytic uremic syndrome. *J Biol Chem*, 284, 15650–8.

Levy, G. G., Nichols, W. C., Lian, E. C., *et al.* (2001). Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature*, 413, 488–94.

Lino, M., Kus, J. V., Tran, S. L., *et al.* (2011). A novel antimicrobial peptide significantly enhances acid-induced killing of Shiga toxin-producing Escherichia coli O157 and non-O157 serotypes. *Microbiology*, 157, 1768–75.

Loirat, C. and Niaudet, P. (2003). The risk of recurrence of hemolytic uremic syndrome after renal transplantation in children. *Pediatr Nephrol*, 18, 1095–101.

Loirat, C., Coppo, P., and Veyradier, A. (2013). Thrombotic thrombocytopenic purpura in children. *Curr Opin Pediatr*, 25, 216–24

Lotta, L. A., Wu, H. M., Mackie, I. J., *et al.* (2012). Residual plasmatic activity of ADAMTS13 is correlated with phenotype severity in congenital thrombotic thrombocytopenic purpura. *Blood*, 120, 440–8.

Lupski, J. R. (2007). Genomic rearrangements and sporadic disease. *Nat Genet*, 39, S43–7.

MacDonald, I. A., Gould, I. M. and Curnow, J. (1996). Epidemiology of infection due to Escherichia coli O157: a 3-year prospective study. *Epidemiol Infect*, 116, 279–84.

Mache, C. J., Acham-Roschitz, B., Fremeaux-Bacchi, V., *et al.* (2009). Complement inhibitor eculizumab in atypical hemolytic uremic syndrome. *Clin J Am Soc Nephrol*, 4, 1312–16.

Maga, T. K., Nishimura, C. J., Weaver, A. E., *et al.* (2010). Mutations in alternative pathway complement proteins in American patients with atypical hemolytic uremic syndrome. *Hum Mutat*, 31, E1445–60.

Magnus, T., Rother, J., Simova, O., *et al.* (2012). The neurological syndrome in adults during the 2011 northern German E. coli serotype O104:H4 outbreak. *Brain*, 135, 1850–9.

Manning, S. D., Motiwala, A. S., Springman, A. C., *et al.* (2008). Variation in virulence among clades of Escherichia coli O157:H7 associated with disease outbreaks. *Proc Natl Acad Sci U S A*, 105, 4868–73.

Manuelian, T., Hellwage, J., Meri, S., *et al.* (2003). Mutations in factor H reduce binding affinity to C3b and heparin and surface attachment to endothelial cells in hemolytic uremic syndrome. *J Clin Invest*, 111, 1181–90.

Martinez-Barricarte, R., Pianetti, G., Gautard, R., *et al.* (2008). The complement factor H R1210C mutation is associated with atypical hemolytic uremic syndrome. *J Am Soc Nephrol*, 19, 639–46.

McGraw, M. E., Lendon, M., Stevens, R. F., *et al.* (1989). Haemolytic uraemic syndrome and the Thomsen Friedenreich antigen. *Pediatr Nephrol*, 3, 135–9.

Mead, P. S. and Griffin, P. M. (1998). Escherichia coli O157:H7. *Lancet*, 352, 1207–12.

Milford, D. (1992) The hemolytic uremic syndromes in the United Kingdom. In B. S. Kaplan, R. S. Trompeter, and J. L. Moake (eds.)

Hemolytic Uremic Syndrome and Thrombotic Thrombocytopenic Purpura, pp. 39–59. New York: Marcel Dekker Inc.
Moake, J. L. (1998). Moschcowitz, multimers, and metalloprotease. *N Engl J Med*, 339, 1629–31.
Moake, J. L. (2002). Thrombotic microangiopathies. *N Engl J Med*, 347, 589–600.
Moore, I., Strain, L., Pappworth, I., *et al.* (2010). Association of factor H autoantibodies with deletions of CFHR1, CFHR3, CFHR4, and with mutations in CFH, CFI, CD46, and C3 in patients with atypical hemolytic uremic syndrome. *Blood*, 115, 379–87.
Morgan, H. P., Schmidt, C. Q., Guariento, M., *et al.* (2011). Structural basis for engagement by complement factor H of C3b on a self surface. *Nat Struct Mol Biol*, 18, 463–70.
Mori, Y., Wada, H., Gabazza, E. C., *et al.* (2002). Predicting response to plasma exchange in patients with thrombotic thrombocytopenic purpura with measurement of vWF-cleaving protease activity. *Transfusion*, 42, 572–80.
Morigi, M., Galbusera, M., Binda, E., *et al.* (2001). Verotoxin-1-induced up-regulation of adhesive molecules renders microvascular endothelial cells thrombogenic at high shear stress. *Blood*, 98, 1828–35.
Morigi, M., Galbusera, M., Gastoldi, S., *et al.* (2011). Alternative pathway activation of complement by Shiga toxin promotes exuberant C3a formation that triggers microvascular thrombosis. *J Immunol*, 187, 172–80.
Morigi, M., Micheletti, G., Figliuzzi, M., *et al.* (1995). Verotoxin-1 promotes leukocyte adhesion to cultured endothelial cells under physiologic flow conditions. *Blood*, 86, 4553–8.
Moschcowitz, E. (1925). Acute febrile pleiochromic anemia with hyaline thrombosis of a terminal arterioles and capillaries. An undescribed disease. *Arch Intern Med*, 36, 89–93.
Moschcowitz, E. (2003). An acute febrile pleiochromic anemia with hyaline thrombosis of the terminal arterioles and capillaries: an undescribed disease. 1925. *Mt Sinai J Med*, 70, 352–5.
Mukhopadhyay, S. and Linstedt, A. D. (2012). Manganese blocks intracellular trafficking of Shiga toxin and protects against Shiga toxicosis. *Science*, 335, 332–5.
Nakajima, H., Kiyokawa, N., Katagiri, Y. U., *et al.* (2001). Kinetic analysis of binding between Shiga toxin and receptor glycolipid Gb3Cer by surface plasmon resonance. *J Biol Chem*, 276, 42915–22.
Nester, C., Stewart, Z., Myers, D., *et al.* (2011). Pre-emptive eculizumab and plasmapheresis for renal transplant in atypical hemolytic uremic syndrome. *Clin J Am Soc Nephrol*, 6, 1488–94.
Nitschke, M., Sayk, F., Hartel, C., *et al.* (2012). Association between azithromycin therapy and duration of bacterial shedding among patients with Shiga toxin-producing enteroaggregative Escherichia coli O104:H4. *JAMA*, 307, 1046–52.
Noris, M., Brioschi, S., Caprioli, J., *et al.* (2003). Familial haemolytic uraemic syndrome and an MCP mutation. *Lancet*, 362, 1542–7.
Noris, M., Caprioli, J., Bresin, E., *et al.* (2010). Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol*, 5, 1844–59.
Noris, M. and Remuzzi, G. (2009). Atypical hemolytic-uremic syndrome. *N Engl J Med*, 361, 1676–87.
Noris, M., Ruggenenti, P., Perna, A., *et al.* (1999). Hypocomplementemia discloses genetic predisposition to hemolytic uremic syndrome and thrombotic thrombocytopenic purpura: role of factor H abnormalities. Italian Registry of Familial and Recurrent Hemolytic Uremic Syndrome/Thrombotic Thrombocytopenic Purpura. *J Am Soc Nephrol*, 10, 281–93.
Nurnberger, J., Witzke, O., Saez, A. O., *et al.* (2009). Eculizumab for atypical hemolytic-uremic syndrome. *N Engl J Med*, 360, 542–4.
O'Brien, A. D., Lively, T. A., Chang, T. W., *et al.* (1983). Purification of Shigella dysenteriae 1 (Shiga)-like toxin from Escherichia coli O157:H7 strain associated with haemorrhagic colitis. *Lancet*, 2, 573.
Ohali, M., Shalev, H., Schlesinger, M., *et al.* (1998). Hypocomplementemic autosomal recessive hemolytic uremic syndrome with decreased factor H. *Pediatr Nephrol*, 12, 619–24.
Orth, D. and Wurzner, R. (2010). Complement in typical hemolytic uremic syndrome. *Semin Thromb Hemost*, 36, 620–4.
Orth, D., Khan, A.B., Naim, A., *et al.* (2009). Shiga toxin activates complement and binds factor H: evidence for an active role of complement in hemolytic uremic syndrome. *J Immunol*, 182, 6394–400.
Ostroff, S. M., Kobayashi, J. M., and Lewis, J. H. (1989). Infections with Escherichia coli O157:H7 in Washington State. The first year of statewide disease surveillance. *JAMA*, 262, 355–9.
Padilla, A., Moake, J. L., Bernardo, A., *et al.* (2004). P-selectin anchors newly released ultralarge von Willebrand factor multimers to the endothelial cell surface. *Blood*, 103, 2150–6.
Palermo, M. S., Exeni, R. A. and Fernandez, G. C. (2009). Hemolytic uremic syndrome: pathogenesis and update of interventions. *Expert Rev Anti Infect Ther*, 7, 697–707.
Palla, R., Lavoretano, S., Lombardi, R., *et al.* (2009). The first deletion mutation in the TSP1-6 repeat domain of ADAMTS13 in a family with inherited thrombotic thrombocytopenic purpura. *Haematologica*, 94, 289–93.
Park, Y. A., Hay, S. N., King, K. E., *et al.* (2009). Is it quinine TTP/HUS or quinine TMA? ADAMTS13 levels and implications for therapy. *J Clin Apher*, 24, 115–19.
Paton, A. W., Srimanote, P., Talbot, U. M., *et al.* (2004). A new family of potent AB5 cytotoxins produced by Shiga toxigenic Escherichia coli. *J Exp Med*, 200, 35–46.
Pechtl, I. C., Kavanagh, D., McIntosh, N., *et al.* (2011). Disease-associated N-terminal complement factor H mutations perturb cofactor and decay-accelerating activities. *J Biol Chem*, 286, 11082–90.
Perez-Caballero, D., Gonzalez-Rubio, C., Gallardo, M. E., *et al.* (2001). Clustering of missense mutations in the C-terminal region of factor H in atypical hemolytic uremic syndrome. *Am J Hum Genet*, 68, 478–84.
Petruzziello-Pellegrini, T. N. and Marsden, P. A. (2012). Shiga toxin-associated hemolytic uremic syndrome: advances in pathogenesis and therapeutics. *Curr Opin Nephrol Hypertens*, 21, 433–40.
Petruzziello-Pellegrini, T. N., Yuen, D. A., Page, A. V., *et al.* (2012). The CXCR4/CXCR7/SDF-1 pathway contributes to the pathogenesis of Shiga toxin-associated hemolytic uremic syndrome in humans and mice. *J Clin Invest*, 122, 759–76.
Peyvandi, F., Lavoretano, S., Palla, R., *et al.* (2008). ADAMTS13 and anti-ADAMTS13 antibodies as markers for recurrence of acquired thrombotic thrombocytopenic purpura during remission. *Haematologica*, 93, 232–9.
Pichette, V., Querin, S., Schurch, W., *et al.* (1994). Familial hemolytic-uremic syndrome and homozygous factor H deficiency. *Am J Kidney Dis*, 24, 936–41.
Pickering, M. C., Cook, H. T., Warren, J., *et al.* (2002). Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. *Nat Genet*, 31, 424–8.
Pickering, M. C., de Jorge, E. G., Martinez-Barricarte, R., *et al.* (2007). Spontaneous hemolytic uremic syndrome triggered by complement factor H lacking surface recognition domains. *J Exp Med*, 204, 1249–56.
Qin, J., Cui, Y., Zhao, X., *et al.* (2011). Identification of the Shiga toxin-producing Escherichia coli O104:H4 strain responsible for a food poisoning outbreak in Germany by PCR. *J Clin Microbiol*, 49, 3439–40.
Remuzzi, G., Galbusera, M., Noris, M., *et al.* (2002b). von Willebrand factor cleaving protease (ADAMTS13) is deficient in recurrent and familial thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *Blood*, 100, 778–85.
Remuzzi, G., Ruggenenti, P., and Bertani, T. (1994). Thrombotic microangiopathy. In C. G. Tisher and B. M. Brenner (eds.) *Renal Pathology: With Clinical and Functional Correlations*, pp. 1154–84. Philadelphia, PA: Lippincott, J.B. Company.
Remuzzi, G., Ruggenenti, P., Codazzi, D., *et al.* (2002a). Combined kidney and liver transplantation for familial haemolytic uraemic syndrome. *Lancet*, 359, 1671–2.
Richards, A., Buddles, M. R., Donne, R. L., *et al.* (2001). Factor H mutations in hemolytic uremic syndrome cluster in exons 18–20, a domain important for host cell recognition. *Am J Hum Genet*, 68, 485–90.
Richards, A., Kemp, E. J., Liszewski, M. K., *et al.* (2003). Mutations in human complement regulator, membrane cofactor protein (CD46),

predispose to development of familial hemolytic uremic syndrome. *Proc Natl Acad Sci U S A*, 100, 12966–71.
Richards, A., Liszewski M. K., Kavanagh, D., *et al.* (2007). Implications of the initial mutations in membrane cofactor protein (MCP; CD46) leading to atypical hemolytic uremic syndrome. *Mol Immunol*, 44, 111–22.
Rivas, M., Miliwebsky, E., Chinen, I., *et al.* (2006). Characterization and epidemiologic subtyping of Shiga toxin-producing Escherichia coli strains isolated from hemolytic uremic syndrome and diarrhea cases in Argentina. *Foodborne Pathog Dis*, 3, 88–96.
Rodriguez de Cordoba, S. (2010). aHUS: a disorder with many risk factors. *Blood*, 115, 158–60.
Roumenina, L. T., Jablonski, M., Hue, C., *et al.* (2009). Hyperfunctional C3 convertase leads to complement deposition on endothelial cells and contributes to atypical hemolytic uremic syndrome. *Blood*, 114, 2837–45.
Ruggenenti, P. and Remuzzi, G. (2011). A German outbreak of haemolytic uraemic syndrome. *Lancet*, 378, 1057–8.
Ruggenenti, P., Galli, M., and Remuzzi, G. (2001). Hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, and antiphospholipid antibody syndromes. In E. G. Neilson and W. G. Couser (eds.) *Immunologic Renal Diseases*, pp. 1179–208. Philadelphia, PA: Lippincott Williams & Wilkins.
Ruggenenti, P., Noris, M. and Remuzzi, G. (2001). Thrombotic microangiopathy, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. *Kidney Int*, 60, 831–46.
Ruggenenti, P., Noris, M., and Remuzzi, G. (2008) Thrombotic microangiopathies. In C. Wilcox (ed.) *Therapy in Nephrology & Hypertension: A Companion to Brenner & Rector's The Kidney*, pp. 294–312. Philadelphia, PA: Saunders Elsevier.
Sadler, J. E. (2008). Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. *Blood*, 112, 11–18.
Safdar, N., Said, A., Gangnon, R. E., *et al.* (2002). Risk of hemolytic uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 enteritis: a meta-analysis. *JAMA*, 288, 996–1001.
Saland, J. M., Emre, S. H., Shneider, B. L., *et al.* (2006). Favorable long-term outcome after liver-kidney transplant for recurrent hemolytic uremic syndrome associated with a factor H mutation. *Am J Transplant*, 6, 1948–52.
Saland, J. M., Ruggenenti, P., and Remuzzi, G. (2009a). Liver-kidney transplantation to cure atypical hemolytic uremic syndrome. *J Am Soc Nephrol*, 20, 940–9.
Saland, J. M., Shneider, B. L., Bromberg, J. S., *et al.* (2009b). Successful split liver-kidney transplant for factor H associated hemolytic uremic syndrome. *Clin J Am Soc Nephrol*, 4, 201–6.
Sanchez-Corral, P., Gonzalez-Rubio, C., Rodriguez de Cordoba, S., *et al.* (2004). Functional analysis in serum from atypical hemolytic uremic syndrome patients reveals impaired protection of host cells associated with mutations in factor H. *Mol Immunol*, 41, 81–4.
Sanchez-Corral, P., Perez-Caballero, D., Huarte, O., *et al.* (2002). Structural and functional characterization of factor H mutations associated with atypical hemolytic uremic syndrome. *Am J Hum Genet*, 71, 1285–95.
Sandvig, K., Olsnes, S., Brown, J. E., *et al.* (1989). Endocytosis from coated pits of Shiga toxin: a glycolipid-binding protein from Shigella dysenteriae 1. *J Cell Biol*, 108, 1331–43.
Saunders, R. E., Abarrategui-Garrido, C., Fremeaux-Bacchi, V., *et al.* (2007). The interactive Factor H-atypical hemolytic uremic syndrome mutation database and website: update and integration of membrane cofactor protein and Factor I mutations with structural models. *Hum Mutat*, 28, 222–34.
Saunders, R. E., Goodship, T. H., Zipfel, P. F., *et al.* (2006). An interactive web database of factor H-associated hemolytic uremic syndrome mutations: insights into the structural consequences of disease-associated mutations. *Hum Mutat*, 27, 21–30.
Schneppenheim, R., Kremer Hovinga, J. A., Becker, T., *et al.* (2006). A common origin of the 4143insA ADAMTS13 mutation. *Thromb Haemost*, 96, 3–6.
Scotland, S. M., Willshaw, G. A., Smith, H. R., *et al.* (1987). Properties of strains of Escherichia coli belonging to serogroup O157 with special reference to production of Vero cytotoxins VT1 and VT2. *Epidemiol Infect*, 99, 613–24.
Scott, E. A., Puca, K. E., Pietz, B. C., *et al.* (2007). Comparison and stability of ADAMTS13 activity in therapeutic plasma products. *Transfusion*, 47, 120–5.
Scully, M., Cohen, H., Cavenagh, J., *et al.* (2007). Remission in acute refractory and relapsing thrombotic thrombocytopenic purpura following rituximab is associated with a reduction in IgG antibodies to ADAMTS-13. *Br J Haematol*, 136, 451–61.
Sellier-Leclerc, A. L., Fremeaux-Bacchi, V., Dragon-Durey, M. A., *et al.* (2007). Differential impact of complement mutations on clinical characteristics in atypical hemolytic uremic syndrome. *J Am Soc Nephrol*, 18, 2392–400.
Siegler, R. L. (1995). The hemolytic uremic syndrome. *Pediatr Clin North Am*, 42, 1505–29.
Siegler, R. L., Obrig, T. G., Pysher, T. J., *et al.* (2003). Response to Shiga toxin 1 and 2 in a baboon model of hemolytic uremic syndrome. *Pediatr Nephrol*, 18, 92–6.
Silberstein, C., Pistone Creydt, V., Gerhardt, E., *et al.* (2008). Inhibition of water absorption in human proximal tubular epithelial cells in response to Shiga toxin-2. *Pediatr Nephrol*, 23, 1981–90.
Singer, K., Bornstein, F. P., and Wile, S. A. (1947). Thrombotic thrombocytopenic purpura; hemorrhagic diathesis with generalized platelet thromboses. *Blood*, 2, 542–54.
Skerka, C., Jozsi, M., Zipfel, P. F., *et al.* (2009). Autoantibodies in haemolytic uraemic syndrome (HUS). *Thromb Haemost*, 101, 227–32.
Soejima, K., Matsumoto, M., Kokame, K., *et al.* (2003). ADAMTS-13 cysteine-rich/spacer domains are functionally essential for von Willebrand factor cleavage. *Blood*, 102, 3232–7.
Stuhlinger, W., Kourilsky, O., Kanfer, A., *et al.* (1974). Letter: Haemolytic-uraemic syndrome: evidence for intravascular C3 activation. *Lancet*, 2, 788–9.
Sugatani, J., Komiyama, N., Mochizuki, T., *et al.* (2002). Urinary concentrating defect in rats given Shiga toxin: elevation in urinary AQP2 level associated with polyuria. *Life Sci*, 71, 171–89.
Sullivan, M., Erlic, Z., Hoffmann, M. M., *et al.* (2010). Epidemiological approach to identifying genetic predispositions for atypical hemolytic uremic syndrome. *Ann Hum Genet*, 74, 17–26.
Tao, Z., Anthony, K., Peng, Y., *et al.* (2006). Novel ADAMTS-13 mutations in an adult with delayed onset thrombotic thrombocytopenic purpura. *J Thromb Haemost*, 4, 1931–5.
Tarr, P. I., Gordon, C. A. and Chandler, W. L. (2005). Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. *Lancet*, 365, 1073–86.
Tarr, P. I., Neill, M. A., Clausen, C. R., *et al.* (1990). Escherichia coli O157:H7 and the hemolytic uremic syndrome: importance of early cultures in establishing the etiology. *J Infect Dis*, 162, 553–6.
Taylor, C. M., Machin, S., Wigmore, S. J., *et al.* (2010). Clinical practice guidelines for the management of atypical haemolytic uraemic syndrome in the United Kingdom. *Br J Haematol*, 148, 37–47.
Thompson, R. A. and Winterborn, M. H. (1981). Hypocomplementaemia due to a genetic deficiency of beta 1H globulin. *Clin Exp Immunol*, 46, 110–19.
Thurman, J. M., Marians, R., Emlen, W., *et al.* (2009). Alternative pathway of complement in children with diarrhea-associated hemolytic uremic syndrome. *Clin J Am Soc Nephrol*, 4, 1920–4.
Trachtman, H., Cnaan, A., Christen, E., *et al.* (2003). Effect of an oral Shiga toxin-binding agent on diarrhea-associated hemolytic uremic syndrome in children: a randomized controlled trial. *JAMA*, 290, 1337–44.
Tsai, H. M. (2010). Pathophysiology of thrombotic thrombocytopenic purpura. *Int J Hematol*, 91, 1–19.
Tsai, H. M. and Lian, E. C. (1998). Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med*, 339, 1585–94.
Tsai, H. M., Rice, L., Sarode, R., *et al.* (2000). Antibody inhibitors to von Willebrand factor metalloproteinase and increased binding of von Willebrand factor to platelets in ticlopidine-associated thrombotic thrombocytopenic purpura. *Ann Intern Med*, 132, 794–9.

Tsai, H. M., Sussman, I. I., and Nagel, R. L. (1994). Shear stress enhances the proteolysis of von Willebrand factor in normal plasma. *Blood*, 83, 2171–9.

Turner, N., Nolasco, L., Tao, Z., *et al.* (2006). Human endothelial cells synthesize and release ADAMTS-13. *J Thromb Haemost*, 4, 1396–404.

Uchida, H., Kiyokawa, N., Horie, H., *et al.* (1999). The detection of Shiga toxins in the kidney of a patient with hemolytic uremic syndrome. *Pediatr Res*, 45, 133–7.

Van Dyck, M. and Proesmans, W. (2004). Renoprotection by ACE inhibitors after severe hemolytic uremic syndrome. *Pediatr Nephrol*, 19, 688–90.

Van Setten, P. A., Monnens, L. A., Verstraten, R. G., *et al.* (1996). Effects of verocytotoxin-1 on nonadherent human monocytes: binding characteristics, protein synthesis, and induction of cytokine release. *Blood*, 88, 174–83.

Van Setten, P. A., van Hinsbergh, V. W., van den Heuvel, L. P., *et al.* (1997). Verocytotoxin inhibits mitogenesis and protein synthesis in purified human glomerular mesangial cells without affecting cell viability: evidence for two distinct mechanisms. *J Am Soc Nephrol*, 8, 1877–88.

Venables, J. P., Strain, L., Routledge, D., *et al.* (2006). Atypical haemolytic uraemic syndrome associated with a hybrid complement gene. *PLoS Med*, 3, e431.

Vesely, S. K., George, J. N., Lammle, B., *et al.* (2003). ADAMTS13 activity in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: relation to presenting features and clinical outcomes in a prospective cohort of 142 patients. *Blood*, 102, 60–8.

Veyradier, A., Obert, B., Houllier, A., *et al.* (2001). Specific von Willebrand factor-cleaving protease in thrombotic microangiopathies: a study of 111 cases. *Blood*, 98, 1765–72.

Warwicker, P., Goodship, T. H., Donne, R. L., *et al.* (1998). Genetic studies into inherited and sporadic hemolytic uremic syndrome. *Kidney Int*, 53, 836–44.

Wilson, C., Torpey, N., Jaques, B., *et al.* (2011). Successful simultaneous liver-kidney transplant in an adult with atypical hemolytic uremic syndrome associated with a mutation in complement factor H. *Am J Kidney Dis*, 58, 109–12.

Wong, C. S., Jelacic, S., Habeeb, R. L., *et al.* (2000). The risk of the hemolytic-uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 infections. *N Engl J Med*, 342, 1930–6.

Zheng, X. L. and Sadler, J. E. (2008). Pathogenesis of thrombotic microangiopathies. *Annu Rev Pathol*, 3, 249–77.

Zheng, X. L., Kaufman, R. M., Goodnough, L. T., *et al.* (2004). Effect of plasma exchange on plasma ADAMTS13 metalloprotease activity, inhibitor level, and clinical outcome in patients with idiopathic and nonidiopathic thrombotic thrombocytopenic purpura. *Blood*, 103, 4043–9.

Zhou, W., Inada, M., Lee, T. P., *et al.* (2005). ADAMTS13 is expressed in hepatic stellate cells. *Lab Invest*, 85, 780–8.

Zimmerhackl, L. B., Hofer, J., Cortina, G., *et al.* (2010). Prophylactic eculizumab after renal transplantation in atypical hemolytic-uremic syndrome. *N Engl J Med*, 362, 1746–8.

Zoja, C., Buelli, S. and Morigi, M. (2010). Shiga toxin-associated hemolytic uremic syndrome: pathophysiology of endothelial dysfunction. *Pediatr Nephrol*, 25, 2231–40.

Zoja, C., Angioletti, S., Donadelli, R., *et al.* (2002). Shiga toxin-2 triggers endothelial leukocyte adhesion and transmigration via NF-kappaB dependent up-regulation of IL-8 and MCP-1. *Kidney Int*, 62, 846–56.