26 The Renal Biopsy

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CHAPTER OUTLINE

KEY POINTS

- Kidney biopsy plays an important role in the management of kidney disease.
- Percutaneous kidney biopsy is generally safe if care is taken to select and prepare the patients beforehand
- Full assessment of the biopsy requires examination by light microscopy, immunohistochemistry and (in most cases) electron microscopy
- The biopsy report should include a morphological description of the biopsy and an interpretation of the appearances in the light of the clinical presentation.
- Recommendations for the essential elements that should be present in the report have been published by the Renal Pathology Society.

INTRODUCTION

The renal biopsy has become a fundamental component in the management of renal disease. Prior to its routine use, only autopsy material was available to investigate the pathophysiology of kidney disease, limiting antemortem diagnosis. However, its development and refinement since the late 1950s have been fundamental for the diagnosis and definition of clinical syndromes and the discovery of new pathological entities.[1] Through the critical analysis of renal biopsies taken at different disease time points, key pathophysiological features of kidney disease have been discovered, which have in turn helped to establish new paradigms in nephrology, and have led to considerable alterations in patient management, with estimates of up to 74% of patients' management changing based on the biopsy results,[2] and in two-thirds of patients, the biopsy revealed unsuspected diagnostic information. This is true for both native renal biopsies and renal transplant biopsies.[3] In addition, much is still being learnt regarding disease pathogenesis through the study of renal biopsy material, which not only remains a "gold standard" for disease diagnosis, but also has allowed the development of novel biopsy markers, which have revolutionized our concepts of pathological mechanisms.

The first percutaneous kidney biopsies were performed over 50 years ago using a liver biopsy needle and intravenous pyelograms for screening, with the patient either sitting or supine. Their success in obtaining renal tissue and in aiding management confirmed the benefit of the procedure.[1] Many innovations, including using real-time ultrasound, which allows visualization of the needle entering the kidney, spring-loaded needles,[4] or needle holders, and careful preoperative evaluation of the patient have improved the rate of obtaining renal tissue while minimizing the risks of the procedure.[5] Consequently, this has placed percutaneous renal biopsy at the very center of modern clinical nephrology. The range of diagnoses for a group of 2219 native kidney biopsies performed at the authors' institution over a 5-year period is shown in Fig. 26.1.

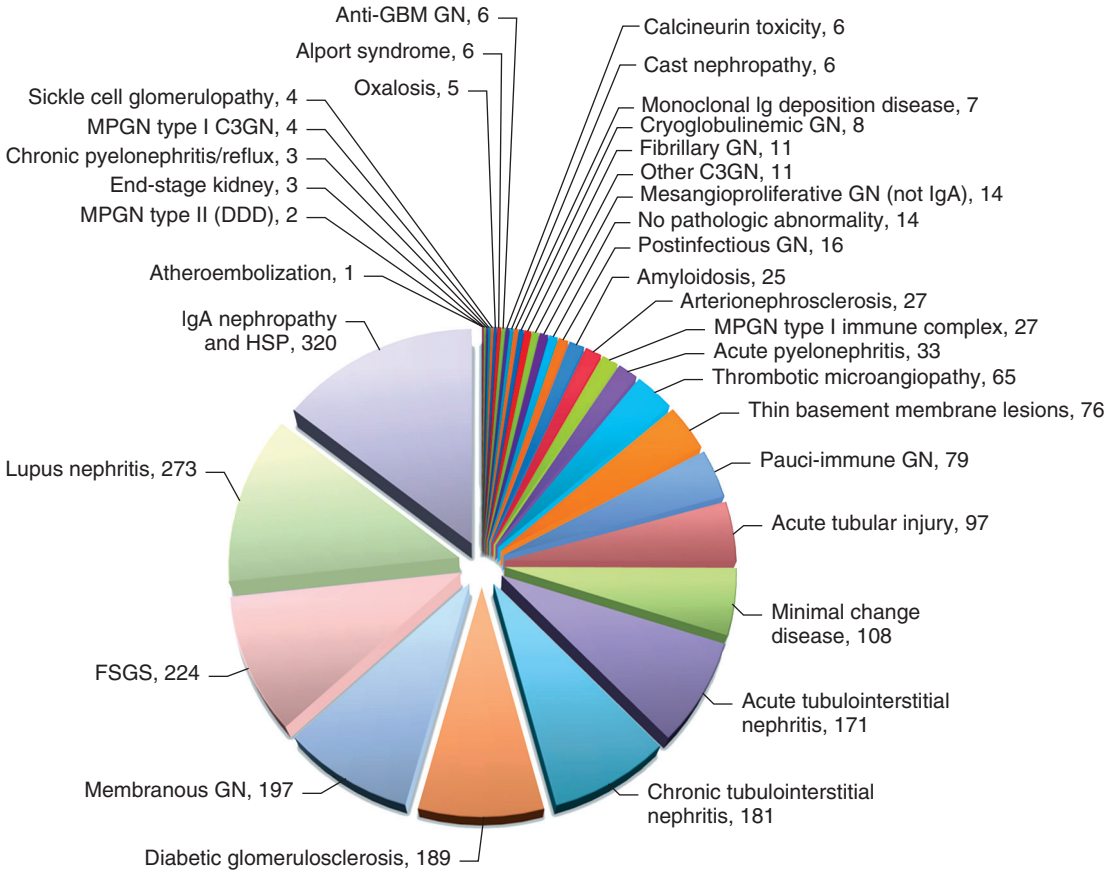

Fig. 26.1 Proportion of diagnoses in 2219 native renal biopsies performed at Hammersmith Hospital over a 5-year period. *DDD,* Dense deposit disease; *FSGS,* focal segmental glomerulosclerosis; *GBM,* glomerular basement membrane; *GN,* glomerulonephritis; *HSP,* Henoch–Schönlein purpura; *Ig,* immunoglobulin; *MPGN,* membranoproliferative glomerulonephritis.

Box 26.1 Indications for Renal Biopsy

- Significant proteinuria (>1 g/day or protein-to-creatinine ratio >100 mg/mmol)
- Microscopic hematuria with any degree of proteinuria
- Unexplained renal impairment (native or transplant kidney)
- Renal manifestations of systemic disease

SAFETY AND COMPLICATIONS OF BIOPSIES

Although generally considered safe, there is a morbidity and a small, but measurable, mortality associated with the procedure, and it is therefore imperative to subject only those patients in whom there will be a potential benefit to these risks. Indications for renal biopsy may vary from one center to another, but accepted indications are listed in Box 26.1. The significant complications related to the procedure are hemorrhage, development of arteriovenous fistulas, and to a lesser extent sepsis.[6–8] Bleeding with macroscopic hematuria and the development of perinephric hematomas may be minor and self-resolving or major and require intervention in the form of blood transfusions, embolization, or rarely surgery. Secondly, there is a risk of formation of arteriovenous fistulas, which may be asymptomatic and spontaneously resolve or lead to a significant vascular steal syndrome, compromising the rest of the kidney through ischemia. Finally, there is the risk of sepsis following the procedure, through the introduction of a septic focus or its dissemination. Overall, the risks of complication vary from center to center and between practitioners, but can be estimated between 3.5% and 13%, with the majority being minor complications (approximately 3%–9%),[6–8] although this appears to have decreased in recent years.[9,10] Mortality from the procedure is generally a result of undiagnosed bleeding with significant hematoma formation and was reported in up to 0.2% of cases from some of the larger biopsy series,[7,8] although other studies suggest that it represents an extremely rare adverse event.[5] Some degree of bleeding is common, as approximately one-half of patients have a drop in hemoglobin postbiopsy, and one-third will develop some hematoma, but only in a minority (≤7%) will bleeding be significant and require intervention.[5,8,11] Complications appear to be more common in native than transplant kidneys, and in patients with more advanced renal impairment, with prolonged bleeding times, or with lower hemoglobin (11 ± 2 vs. 12 ± 2 g/dL).[7,8] One prospective study identified the only risk factors for bleeding complications as being female, younger patients (35 ± 14.5 years vs. 40.3 ± 15.4 years), and those with a prolonged partial thromboplastin

time.[12] Interestingly, needle size, number of passes, blood pressure, and renal impairment were not different between those with bleeding complications and those without. However, in this study all patients with prolonged bleeding times received DDAVP (1-deamino-8-D-arginine vasopressin) to correct the abnormality and 75% of patients had serum creatinine of less than 132 μmol/L. Conversely, others using retrospective univariate analysis have reported that blood pressure of 160/100 mm Hg or higher or a serum creatinine of greater than 2 mg/dL more than doubled the risk of bleeding.[8,13] Overall, however, no effective means has been established to identify those individuals at risk of developing "clinically" significant complications. In one small series, postbiopsy ultrasound within an hour had a 95% negative predictive value for predicting clinically significant hemorrhagic complications,[11] meaning that the absence of a hematoma on the postbiopsy scan was very suggestive of an uncomplicated clinical course. Debate continues regarding the routine use of DDAVP to counteract uremic bleeding tendencies. In part this is because its use was previously reserved for only those patients with prolonged bleeding times, and numerous studies have since demonstrated that complication rates are no different if bleeding time estimation is omitted from the preoperative assessment,[14,15] as it does not predict clinical complications.[12] However, more recent data from a randomized double blinded trial suggested a significant benefit in preventing bleeding complications with few adverse events.[16] A total of 162 low-risk adult patients undergoing biopsy were enrolled and randomized to subcutaneous DDAVP (0.3 μg/kg) or placebo. The patients were normotensive, had preserved renal function with serum creatinine of less than 1.5 mg/dL (estimated glomerular filtration rate >60 mL/min), and demonstrated a significant reduction in postbiopsy bleeds from 30.5% to 13.7% (relative risk 0.45), a significant reduction in hematoma size in those who did bleed, and a reduction in duration of hospital stay. However, hemoglobin drop after biopsy was minimal and there were no major complications, leading some to question the benefit of reduction in clinically unimportant hematomas, which can be frequently found following biopsy if looked for. No thrombotic, hyponatremic, or cardiovascular events were recorded. Whether these data, in patients with preserved renal function, could be translated to those higher-risk patients with greater renal impairment is unclear and is a question worthy of a randomized trial.

Many centers stop antiplatelet therapy prebiopsy for elective procedures, but recent data suggest that bleeding rates may be no different in those taking aspirin or stopping a week beforehand, and may avoid the increased risk of cardiovascular events following aspirin withdrawal.[9] In a center that does not routinely stop aspirin (but does stop clopidogrel), a retrospective analysis of 2563 biopsies revealed a major bleeding complication in only 2.2%, and in those in whom a complete drug record was available, no significant difference was noted on or off aspirin (357 vs. 1509, respectively, $p = .93$).[10] Very limited data are available on biopsies performed on patients taking clopidogrel.[17]

Guidelines on consenting patients and providing appropriate risk estimates have been produced by certain national renal groups and one such example is provided in Table 26.1. These estimates may err on the conservative side and should be adapted to local practice if adequate complication data are available. As well as developing procedure-related complications, there is the chance that an inadequate core of tissue is obtained for diagnosis, containing too few glomeruli or insufficient cortical material, and this is reported in between 1% and 5% of cases. The size requirements for accurate diagnosis are discussed later.

Table 26.1 Quoted Risks of Renal Biopsy[a]

Complication	Quoted Risk
Macroscopic hematuria	1:10
Bleeding that requires a blood transfusion	<1:50
Bleeding that may require urgent x-ray tests or even an operation to stop the bleeding	<1:1500
Severe bleeding necessitating nephrectomy	<1:3000
Deaths	Extremely rare

[a]According to UK Renal Association (http://www.renal.org/information-resources/procedures-for-patients).

Table 26.2 Contraindications to Renal Biopsy

Absolute Contraindications	Relative Contraindications
Uncontrolled hypertension	Single kidney
Bleeding diathesis	Antiplatelet/clotting agents[a]
Widespread cystic disease	Anatomical abnormalities
Hydronephrosis	Small kidneys
Uncooperative patient	Active urinary/skin sepsis
	Obesity[a]

[a]Aspirin and body mass index >40 kg/m^2 were not found in recent limited cohort data to be a significant risk.

There are certain absolute contraindications, which preclude percutaneous biopsy, whereas there are a number of relative contraindications (Table 26.2) that may be circumvented depending on the importance of the biopsy, the operator's experience, and the supportive facilities available. Ideally, all efforts should be made to deal with the relative contraindications; however, in the context of acute renal failure this may not always be possible. With modern techniques evidence is emerging that previously perceived high-risk factors such as obesity, plasma cell dyscrasias such as myeloma, or amyloidosis are not actually associated with higher rates of bleeding complications.[10,18,19] The critical preoperative steps are to ensure that blood pressure is controlled, that the patient does not have a bleeding diathesis or a urinary tract infection, and that the kidneys are suitably imaged, with no evidence of obstruction, widespread cystic disease, or malignancy (although percutaneous biopsy is increasingly used to diagnose the nature of renal masses). As a result, preoperative assessment should allow those patients unsuitable for percutaneous biopsy to be referred for an alternative approach (Fig. 26.2). In these patients, there are other means of obtaining renal tissue, which include open biopsies,[20] laparoscopic biopsies, or transjugular biopsies.[21] Each is associated with certain complications and has particular merits depending on the clinical scenario (Table 26.3). Overall,

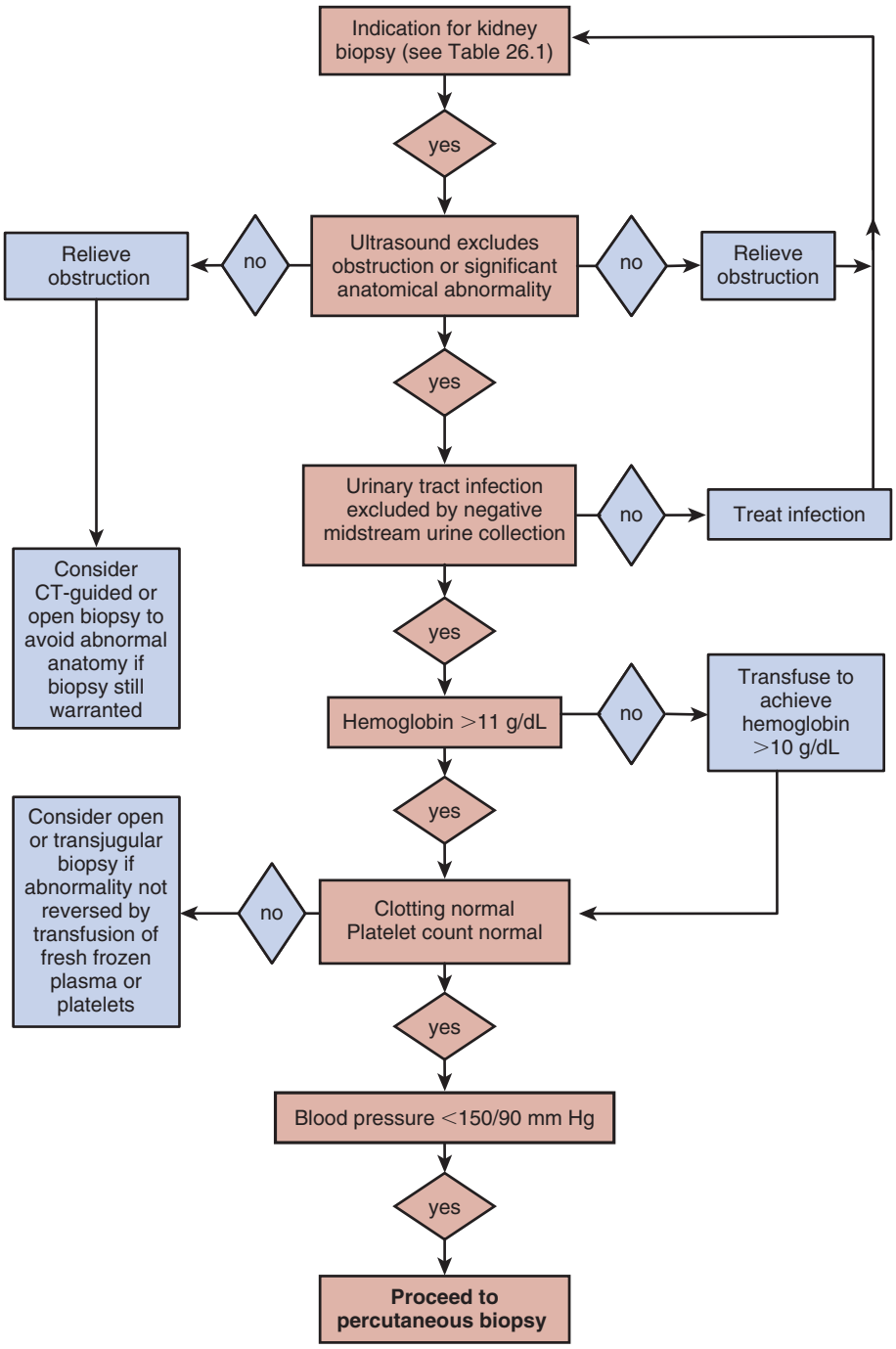

Fig. 26.2 Renal biopsy flowchart. *CT,* Computed tomography.

these are generally only required for a minority of potential biopsy patients.

The safe duration for observation following renal biopsy has been investigated in a number of studies, which suggest that early discharge (after only 4-hour observation) will result in a number of missed complications, with many more occurring between 8 and 24 hours postprocedure. Even after 8 hours, 23% to 33% of complications will be missed. However, an overnight stay will allow an extra 20% of complications to be identified prior to discharge with between 85% and 95% of complications being identified at 12 hours and 89% and 98% following 24-hour observation.[7,22] Some units practice a policy of day biopsies with a minimum 6-hour bed rest period, which is extended only if there is evidence of bleeding, and this appears to be associated with no increased complication rates.[23] Vigilant observation of blood pressure, pulse rate, and evidence of hematuria is required in all cases.

BIOPSY HANDLING

Detailed descriptions of methods of handling biopsies can be found in a number of publications including Churg and colleagues,[24] Furness,[25] and Walker and colleagues.[26]

Table 26.3 Alternative Methods for Obtaining Renal Tissue and Their Risks and Benefits Compared With a Percutaneous Approach

Method	Advantage	Disadvantage
Transjugular approach	Can be of use in those with a bleeding diathesis, ventilated patients, or if combined liver and renal biopsy is required	Risk of capsular perforation Inadequate material in up to 24%
Open approach	High yield of adequate tissue Hemostasis is more secure	Requires general/spinal anesthesia; longer recovery period
Laparoscopic approach	High yield of adequate tissue Hemostasis is more secure	Requires general/spinal anesthesia; longer recovery period

A full assessment of the renal biopsy requires examination by light microscopy, immunohistochemistry, and electron microscopy (EM), with the use of other tests in some circumstances. Therefore it is necessary for the biopsy to be divided to provide material for each of these methods of examination. During this process it is extremely important that the biopsy is not damaged by handling, or by drying, and that the tissue is fixed in an appropriate fixative as quickly as possible, ideally within minutes. This is best achieved by dividing the biopsy at the bedside. Examination of the biopsy with a dissecting microscope allows cortex, containing glomeruli, to be distinguished from medulla and thus facilitates assessment of the adequacy of the cores and division of the biopsy so that glomeruli are present in the samples for each modality of examination. If a dissecting microscope is not available, then a standard light microscope can be used with the biopsy placed in a drop of normal saline on a microscope slide. If it is not possible to examine the biopsy in this way, then a standard approach to obtain material for EM is to take small fragments (approximately 1 mm in length) from each end of each core. In that way if there is cortex in the core, glomeruli should be sampled. The remainder of the cores can then be divided for light microscopy and for immunofluorescence (IF). The part of the biopsy for light microscopy is then placed in appropriate fixative and that for IF is either snap frozen or transported to the laboratory in a suitable transport medium such as that described by Michel et al.[27]; tissue placed in this medium can remain for several days at room temperature without loss of antigens. During division of the biopsy it is important not to introduce artifacts due to crushing or stretching. Forceps should not be used to pick up the specimen; this can be done using either a needle or a small wooden stick such as a toothpick. The biopsy should be cut using a fresh scalpel.

If the biopsy has to be taken to the histology laboratory for division, this should be done as quickly as possible with the biopsy wrapped in saline-moistened gauze or in tissue culture medium. Artifacts may be produced if the biopsy is placed on dry gauze or gauze moistened with water, or if it is placed in ice-cold saline.

If the amount of material obtained at biopsy is limited, then it may be necessary to adapt the way in which it is divided and the decision as to how this is done must depend on the clinical question. In most cases it is possible to omit frozen material for IF and instead perform immunohistochemistry on paraffin sections. However, if there is a suspicion of crescentic glomerulonephritis due to antiglomerular basement membrane (anti-GBM) disease, IF is more reliable for detecting the linear capillary wall staining. It may be possible to omit EM and perform it if necessary on material reprocessed from the paraffin block, but if this is done, it is not possible to obtain accurate measurements of glomerular capillary membrane thickness.[28]

LIGHT MICROSCOPY

The most commonly used fixative for light microscopy is buffered 10% aqueous formaldehyde solution. This is actually a 10% solution of the 37% commercially available concentrated solution of formaldehyde, giving a final concentration of about 4%. This fixative is generally available in all histology laboratories, provides adequate fixation for light microscopy, and also allows the tissue to be used for immunohistochemistry and EM. Some more specialized fixatives such as Bouin or Zenker fixative provide better preservation of certain morphologic details, but in general the problems with handling these fixatives, and the difficulties of subsequently using the material for immunohistochemistry or EM, outweigh the advantages. For example, Bouin contains picric acid that is explosive when dry. However, we do commonly use Bouin fixative for examination of mouse kidneys where the improvement in glomerular morphology is significant. Methacarn, a modified Carnoy fixative, also provides good fixation for light microscopy and EM and may allow the immunohistochemical detection of antigens that are not detected in formalin-fixed tissue. Details of the preparation of various fixatives can be found in the appendix of Churg and colleagues.[24]

The standard method of processing tissue for light microscopy is by dehydration in graded alcohols, transfer to a clearing agent such as xylene, and embedding in paraffin wax. This is usually performed in an automated instrument but can be done by hand. Rapid processing schedules allow for same-day processing and it is possible to obtain stained slides within 3 to 4 hours of receipt of the specimen in the laboratory.

It is important to have thin uniform sections for light microscopy. These should be cut as thin as possible – no greater than 3 μm. It is often stated that renal biopsy sections should be cut at 2 μm, but this may lead to problems in cutting with damage to the tissue. Since many pathologic lesions may be focal within glomeruli, interstitium, or vessels, it is essential that the biopsy is examined at multiple levels and each laboratory will have their preferred way to achieve that. In general, serial sections should be cut with at least two placed on each slide. Multiple slides can then be stained with each stain, with some intervening unstained sections kept either for potential immunohistochemical examination or for other special stains as necessary.

STAINING FOR LIGHT MICROSCOPY

Most renal pathologists employ a number of stains for light microscopy. The commonly used stains are hematoxylin and eosin (H&E), periodic acid–Schiff (PAS) reaction, silver methenamine, and a trichrome stain. The H&E stain is a good general histological stain for studying the overall architecture of the kidney. It is good for studying the morphology of tubular cells and the morphology of interstitial infiltrates. With experience the different staining characteristics of hyaline, fibrin, and amyloid, all of which are eosinophilic, can usually be distinguished. However, the H&E stain does not distinguish staining of glomerular matrix and basement membrane from cell cytoplasm, and therefore is less useful for the assessment of glomerular architecture. In the PAS reaction the mesangial matrix and basement membrane are stained purple and this allows a good assessment of the amount of matrix and the thickness of the GBM. PAS also stains the tubular basement membranes and hyaline deposits. The silver methenamine stain is the best stain for studying the detailed morphology of the GBM and for highlighting the membrane spikes seen in membranous glomerulonephritis and the double contours seen in membranoproliferative glomerulonephritis. Its only drawback is that a satisfactory result is more technically demanding than the other stains. A trichrome stain, such as Masson trichrome, will stain the glomerular mesangial matrix and basement membrane and may also help in highlighting fibrin and immune complex deposits. Other stains are a matter of personal preference. We always use an elastin stain to demonstrate the elastic laminae of vessels and this is counterstained with picrosirius red to stain fibrillar collagen in the interstitium. Amyloid is most specifically detected in a Congo red stain and we feel it is prudent to perform this in all native biopsies. This is the exception to the requirement for thin sections; since the Congo red stain is relatively insensitive, a section cut at 10 μm should be used. Details of staining methods are given in the appendix of Churg and colleagues.[24] Other stains that may be employed when necessary include the von Kossa stain that demonstrates calcium deposition and the Perls Prussian blue stain for iron.

EXAMINATION OF THE BIOPSY BY LIGHT MICROSCOPY

It is important to approach the examination of the biopsy systematically. Sections should first be assessed at low power to determine what parts of the kidney (or other structures in some cases) they contain, including whether there is cortex and/or medulla. A low-power view will also allow an assessment of the amount of chronic nephron damage, as demonstrated by tubular atrophy and interstitial fibrosis, and the presence of interstitial inflammatory infiltrates. It will also allow an assessment of interstitial expansion, most commonly due to either edema or fibrosis, but occasionally due to infiltration by, for example, amyloid. Examination should then proceed by studying the glomeruli, tubules, interstitium, and vessels, including arteries, arterioles, and veins, in more detail. Features that should be looked for in glomeruli and tubules are detailed in Boxes 26.2 and 26.3. Arterioles should be examined for the presence of hyalinosis, thrombosis, and necrosis. Arteries should be assessed for intimal thickening and whether it is accompanied by reduplication of the internal elastic lamina, thrombosis, necrosis, inflammation, and cholesterol emboli.

Box 26.2 Features to Be Assessed by Light Microscopy in Glomeruli

- Size.
- Cellularity: if increased, then are the extra cells mesangial, in capillary lumens (endocapillary), or in Bowman's space? (NB: Normal mesangial areas contain two to three cells.)
- Capillary wall thickness (use periodic acid–Schiff or silver stain); if thickened, are there double contours or spikes on the silver stain?
- Is the mesangium expanded? If so, are there nodules?
- Is there deposition of abnormal material (e.g., amyloid)?
- Is there segmental sclerosis?
- Is there thrombosis?
- Is there necrosis?

Box 26.3 Features to Be Assessed by Light Microscopy in Tubules

- Percentage atrophy
- Signs of acute damage (e.g., dilatation, epithelial flattening, granular casts, mitoses)
- Tubulitis
- Casts: Granular casts suggest acute tubular injury; eosinophilic fractured casts suggest myeloma; neutrophil casts suggest acute pyelonephritis
- Crystals (e.g., oxalate)
- Viral inclusions (e.g., BK virus)

TERMINOLOGY IN DESCRIPTION OF GLOMERULAR DISEASE

The involvement of glomeruli by a pathological process can be defined by the percentage of glomeruli involved by a lesion and by whether the lesion involves all or only part of any individual glomerulus. A lesion that involves all or nearly all glomeruli is described as "diffuse," whereas one that involves some but not all glomeruli is described as "focal." In the definitions given in the World Health Organization (WHO) atlas of glomerular diseases, it was suggested that the cutoff for focal versus diffuse should be 80% of glomerular involvement. However, in recent classifications of lupus glomerulonephritis[29] and immunoglobulin A (IgA) nephropathy,[30] the cutoff is defined as 50%. If a lesion involves only part of a glomerulus, that is, with some capillary lumens remaining uninvolved, it is called "segmental," whereas if it involves the whole glomerulus, it is called "global." In the classifications of lupus glomerulonephritis[29] and IgA nephropathy,[30] the cutoff is set at 50% glomerular tuft involvement, except for segmental sclerosis in IgA nephropathy, in which any area of sclerosis that leaves some of the glomerulus unaffected is defined as segmental.

Box 26.4 Definitions of Terms Used in Describing Glomerular Lesions

Sclerosis: A lesion resulting from an increase in mesangial matrix and/or collapse and condensation of the basement membranes – the sclerotic material stains with eosin, periodic acid–Schiff (PAS), and silver stains.

Hyalinosis: Lesion containing an acellular structureless material consisting of glycoproteins and sometimes lipids – stains intensely with eosin and PAS but not with silver stains.

Fibrosis: A lesion consisting of collagen fibers, which may be differentiated from sclerosis by not staining with PAS reagent or silver stains.

Necrosis: A lesion characterized by fragmentation of nuclei and/or disruption of the basement membrane, often associated with the presence of fibrin-rich material.

Extracapillary proliferation or cellular crescent: Extracapillary cell proliferation of more than two cell layers with >50% of the lesion occupied by cells.

Extracapillary fibrocellular proliferation or fibrocellular crescent: An extracapillary lesion comprising cells and extracellular matrix, with <50% cells and <90% matrix.

Extracapillary fibrosis or fibrous crescent: >10% of the circumference of Bowman's capsule covered by a lesion composed of >90% matrix.

There are a number of other terms, such as sclerosis and hyalinosis, that have specific definitions in the glomerulus and these are listed in Box 26.4.

IMMUNOHISTOCHEMISTRY

The understanding of renal pathology was transformed in the 1960s by the use of IF microscopy. This allowed the detection and localization of immunoglobulins and complement components in glomeruli, and the identification of new entities such as IgA nephropathy. It is mandatory to perform immunohistochemistry for a full assessment of glomerular pathology in biopsies for native kidneys. The use of immunohistochemistry in transplant biopsies is discussed further later. There are a number of diagnoses that cannot be made on renal biopsy without immunohistochemistry including IgA nephropathy, C3 glomerulopathy (GN), C1q nephropathy, anti-GBM disease, and light-chain deposition disease.

In native kidneys a minimum panel of immunohistochemical stains would include antibodies for IgA, IgG, IgM, C3c, and kappa and lambda light chains. Light-chain immunohistochemistry is very important if diagnoses such as light-chain deposition disease, monoclonal immunoglobulin deposition disease, or proliferative glomerulonephritis with monoclonal immunoglobulin deposits are not to be missed. Many pathologists would also add antibodies for C1q, C4c, and fibrinogen to this routine panel. In transplant kidney biopsies staining for C4d is invaluable in assessing the activation of the classical pathway of complement by antibody and hence in the diagnosis of antibody-mediated rejection. There are a number of other antigens whose detection may be useful in particular circumstances. These include:

1. Microorganisms, including BK virus, cytomegalovirus, and Epstein–Barr virus.
2. Amyloid proteins. Antibodies are available to AA amyloid and many of the rarer inherited forms of amyloid.
3. Alpha chains of type 4 collagen. In suspected hereditary nephropathy of Alport type, it may be helpful to stain for the alpha 3 and alpha 5 chains of type IV collagen.
4. IgG subclasses in cases of suspected monoclonal immunoglobulin deposition.
5. Myoglobin in suspected myoglobinuria.
6. Lymphocyte surface antigens particularly in cases of suspected lymphoid neoplasia.
7. Type III collagen in collagenofibrotic GN.
8. Fibronectin in fibronectin GN.

Immunohistochemistry is performed either on cryostat sections of a piece of snap-frozen tissue or on paraffin sections. Antigen detection on frozen sections is usually performed using an antibody labeled with a fluorochrome and this is then viewed using a fluorescence microscope – commonly referred to as immunofluorescence or IF. The use of fluorescent-labeled antibodies on frozen sections is technically straightforward and very sensitive because the antigens have not been altered by fixation. There are some drawbacks. First, it requires a separate piece of tissue to have been obtained at the time of biopsy. Second, the morphology of frozen sections is never as good as paraffin sections and so it may be more difficult to define the site of the antigen within the glomerulus. In addition, immunofluorescent sections will fade over time but if appropriately mounted and refrigerated in the dark, they will retain staining for weeks to months.

If paraffin sections are used, then some form of antigen retrieval is essential for most antigens because they become "masked" during fixation and processing. For the detection of immunoglobulins and complement the antigen retrieval that works best is some form of protease digestion. The length of time required for protease digestion is critically dependent on a number of factors such as the length of time the biopsy has been in fixative and the particular processing schedule used; some of these may be difficult to control. This variability of the antigen retrieval process is the major drawback of immunohistochemistry on paraffin sections and means that results are highly dependent on the skills of the technician performing the staining. After the antigen retrieval step, antigens are generally detected using a primary antibody followed by a detection system that leads to the deposition of a colored reaction product that is visible by light microscopy. Commonly this product is developed by a reaction that uses the enzyme horseradish peroxidase, and hence this method is often referred to as "immunoperoxidase" staining. However, it is also possible to use fluorescent antibody staining on paraffin sections after antigen retrieval.[31]

The major advantage of immunohistochemistry on paraffin sections is that it is not necessary to take a separate piece of tissue for frozen section. In addition, it is possible to specifically localize antigens and compare this with adjacent sections examined by light microscopy. However, it is technically demanding and also it is significantly less sensitive for some antigens. Direct comparison of the two methods showed that detection of IgG, IgA, and C3c by immunoperoxidase on protease-digested deparaffinized sections of formaldehyde-fixed tissue is, with few exceptions, equal to IF on frozen sections.[32] Our experience is that it is extremely difficult to get satisfactory staining for light chains

using peroxidase techniques on paraffin sections (although fluorescence on paraffin sections may be more successful) and that detection of the linear capillary wall staining of anti-GBM antibodies is more difficult in paraffin sections. It may also be more difficult to detect very early deposits of membranous glomerulonephritis in paraffin as compared with frozen tissue.

In our experience most renal pathologists find IF on frozen sections the most satisfactory way to detect immunoglobulins and complement but, regardless of preference, there will always be cases in which no material is available for frozen section, or the material is inadequate, and so laboratories should also be competent to carry out immunohistochemistry on paraffin sections.

In reporting immunohistochemical staining for immunoglobulins and complement, it is important to describe the site of staining in the glomerulus (e.g., mesangial or capillary wall), its nature (whether linear, finely or coarsely granular), and its intensity. For estimation of intensity most pathologists rely on semiquantitative subjective scale from 0 to 3+, but formal quantitation by image analysis may be useful for research. In addition to the glomerulus, staining should also be assessed in the tubules, especially the tubular basement membrane, interstitium, and vessels.

ELECTRON MICROSCOPY

EM is invaluable for assessing structural changes in the glomerulus and for identifying immune complexes, which are seen as areas of electron density. Although the importance of EM has become much reduced in other areas of surgical pathology, because of the development of immunohistochemistry, it remains an invaluable technique for the examination of glomeruli in biopsies in native kidneys and, increasingly, for the determination of causes of dysfunction in transplant kidneys. The part of the renal biopsy on which EM is to be performed is usually placed in separate fixative, although entirely satisfactory results can be obtained using material fixed in formalin. Most laboratories prefer either ice-cold glutaraldehyde or paraformaldehyde. The material is then exposed to osmium tetroxide and processed into resin blocks. In order to select the areas to be studied, "semithin" 0.5-µm sections are first screened by light microscopy to select areas of interest that can then be examined further on the electron microscope. An ultramicrotome is then used to obtain the very thin sections required for the electron microscope. A permanent record of the electron microscopic appearances is kept either as photographs or, increasingly, as digital images. As with light microscopy, examination of the biopsy by EM should be systematic with assessment of the glomerular capillary basement membrane and its thickness; the endothelium and whether there is thickening or loss of fenestrations; the capillary lumen and particularly whether there is narrowing by cells or other material; the podocytes looking particularly at the preservation of the foot processes and whether the cell bodies show any vacuolation or microvillous change. The presence of any electron-dense deposits – most commonly due to immune complex deposition – should be noted together with their distribution – mesangial, subendothelial, or subepithelial. EM may also demonstrate a number of other structures such as fibrils in amyloidosis or fibrillary glomerulonephritis, tubules in immunotactoid GN, or the characteristic inclusion bodies of various storage diseases.

Although EM is most useful in the assessment of glomerular morphology, it may also be very helpful in demonstrating ultrastructural changes in other parts of the kidney. For example, it may help in demonstrating tubular basement membrane immune complexes, in elucidating the nature of tubular epithelial cell inclusions, and in examining the morphology of mitochondria in tubular epithelial cells, which may show abnormalities in inherited conditions or as a result of drugs.

There have been several studies that have assessed the utility of EM in the assessment of native kidney biopsies. Most studies suggest that EM provides useful information in about half of all native kidney biopsies and is essential for diagnosis in about 20%.[33] Because it is impossible to know which these are at the time of biopsy, it is prudent to always have material available for EM even if, in some cases, it is not processed further after light microscopy and immunohistochemistry. Box 26.5 lists some conditions for which EM is essential for the diagnosis and others where it is helpful. Also listed are some conditions in which the diagnosis may be reached without EM but, even in these cases, it is important to remember that EM may allow a more detailed description of the morphology of these conditions or also reveal a totally unrelated pathology.

Box 26.5 Examples of the Use of Electron Microscopy in Diagnosis of Kidney Biopsies

Electron Microscopy (EM) Essential for Diagnosis

Thin basement membrane lesion
Fibrillary glomerulopathy (GN)
Immunotactoid GN
Alport syndrome
Fabry disease
Lecithin-cholesterol acetyltransferase deficiency
Nail patella syndrome

EM Very Helpful

Dense deposit disease
Minimal change disease
Early diabetic GN
Early membranous GN (particularly if only paraffin sections are available for immunohistochemistry)
Membranoproliferative GN
Postinfectious GN
Human immunodeficiency virus (HIV) nephropathy
Lipoprotein GN
Collagenofibrotic GN

Diagnosis May Be Made Without EM

IgA nephropathy
Acute tubulointerstitial nephritis
Myeloma cast nephropathy
Pauci-immune crescentic GN
Amyloid (although amyloid fibrils may be detected by EM when it has been missed on light microscopy)

Morphometric analysis of EM is mainly of importance for research. However, it is important to be able to measure the thickness of the GBM in order to quantitate the thinning that may be seen in thin basement membrane lesion or the thickening commonly seen in diabetic glomerulosclerosis. Accurate unbiased measurement of the GBM thickness requires complex morphometric techniques to avoid the bias introduced by tangential sectioning of capillary loops. However, in practice it is satisfactory to use direct measurement of GBM thickness (distance from endothelial to podocyte plasma membrane) and determination of the arithmetic mean of such measurements. Das et al.[34] found that if 16 measurements from each of two glomeruli were made using this direct method, the results were reproducible. Ideally, each laboratory should define a normal range using this method.

OTHER STUDIES ON THE RENAL BIOPSY

In addition to examination by light microscopy, immunohistochemistry, and EM it may also be appropriate to consider other methods for studying the tissue. In cases of suspected infection, part of the biopsy may be sent for culture or for polymerase chain reaction for infective organisms. In biopsies with lymphoid infiltrates, immunoglobulin gene rearrangement studies may allow the confirmation of clonality. The chemical composition of material in the biopsy (e.g., crystalline material) may be determined by energy-dispersive x-ray spectroscopy.

There has been considerable interest in the possibilities of extracting messenger RNA (mRNA) from biopsies, in order to study differences in gene expression in different pathological conditions,[35] and in studying the range of proteins in the biopsy – the proteome.[36] These techniques have been applied to whole biopsies or to parts of the biopsy – for example, glomeruli isolated either by simple dissection under a dissecting microscope or by laser capture microdissection. Examination by mass spectrometry may allow identification of the fibril proteins of amyloid or fibrillary glomerulonephritis.[37,38] Examination of mRNA transcripts shows considerable promise for assisting in the diagnosis of kidney transplant rejection.[39]

BIOPSIES OF TRANSPLANTED KIDNEYS

The handling of transplant biopsies differs in some respects from that of native kidneys. For biopsies taken to assess the cause of kidney dysfunction in the first few months after transplantation, it may not be necessary to carry out immunohistochemistry with a full panel of antibodies to immunoglobulins and complement, or to perform EM, unless there is a clinical suspicion of glomerular disease. However, immunohistochemistry for C4d is performed to assess antibody binding and complement activation on peritubular capillary endothelium. In later biopsies EM is very useful in the diagnosis of chronic allograft GN and its differentiation from recurrence of de novo glomerulonephritis. It is also helpful in identifying chronic rejection involving peritubular capillaries, which is associated with multilayering of the peritubular capillary basement membranes.[40] The recommendations from the Banff Conference on Allograft Pathology 2013[41] are that ultrastructural studies should be performed in all biopsies from patients who are sensitized, have documented donor-specific antibodies at any time after transplantation, and/or who have had a prior biopsy showing C4d staining, glomerulitis, and/or peritubular capillaritis. It is also advised that EM be considered in all biopsies performed more than 6 months after transplantation and in "for-cause" biopsies done more than 3 months after transplantation to determine if early changes of transplant GN are present, prompting testing for donor-specific antibodies.

THE SIZE OF THE BIOPSY

The renal biopsy is only a small sample of the renal parenchyma and this always needs to be kept in mind when making inferences about the state of the whole kidney from changes seen in the biopsy. Some diseases may only affect the kidney focally and therefore may be missed on biopsy, for example, reflux nephropathy or arterial cholesterol emboli. Others may be segmental at the level of the glomerulus, for example, focal and segmental glomerulosclerosis or pauci-immune necrotizing glomerulonephritis, and therefore the chance of detecting them will depend on how many glomeruli are present in the biopsy and how many sections are examined. Sampling is also a problem when we make inferences from the amount of disease we see in the biopsy to the amount that affects the kidney. For example, if 20% of the glomeruli in a biopsy have crescents, we tend to assume that this is the percentage of the glomeruli in the kidney that have crescents. However, because of the small size of most biopsies, the confidence limits we can place on the true involvement of glomeruli are usually very wide. An elegant mathematical description of the problems of glomerular sampling has been published by Corwin et al.[42] This shows, for example, that to confidently exclude a segmental glomerular disease that is affecting about 5% of the glomeruli, a biopsy with 20 glomeruli is needed. The situation is worse if we consider the problem of comparing the amount of glomerular involvement in two different biopsies, a question that often arises, for example, in patients with lupus glomerulonephritis who have repeat biopsies. In that case, to confidently detect a 10% difference in glomerular involvement between two biopsies would require over 100 glomeruli in each biopsy. To detect differences of 25% to 40% glomerular involvement the minimum biopsy size is 20 to 25 glomeruli.

For some diseases, classification schemes have defined minimum sizes for biopsy adequacy. Thus, for lupus glomerulonephritis it is suggested that a biopsy should contain a minimum of 10 glomeruli.[29] In transplant biopsies the Banff group has suggested that the requirements for biopsy adequacy are 10 or more glomeruli with at least two arteries.[43] It has been shown that examining two rather than one core of tissue increases the sensitivity for the diagnosis of acute rejection from 91% to 99%.[44] In acute cellular rejection, examining slides taken at only one level, rather than at three, misses 33% of cases with intimal arteritis.[45]

THE BIOPSY REPORT

The biopsy report should include a morphologic description of the biopsy and an interpretation of the appearances in

the light of the clinical presentation. The changes seen on light microscopy, immunohistochemistry, and EM must be integrated and this is best done if a single person examines the biopsy by each modality. A Committee of the Renal Pathology Society has published recommendations for the essential elements that should be present in a renal biopsy report.[46]

The description of the light microscopy should include the number of glomeruli present and the number that show global or segmental sclerosis. It is essential to provide a quantitative estimate of the amount of irreversible nephron damage in the biopsy and, where appropriate, the severity of any active inflammatory process. The best way to estimate the irreversible damage is by specifying the number of globally sclerosed glomeruli and the amount of tubular atrophy and interstitial fibrosis. The estimate of activity will depend on the particular disease process but should include an indication of the proportion of glomeruli involved by crescents, necrosis, and endocapillary hypercellularity. For some diagnoses there are established classification schemes that should be applied to the biopsy, for example, the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) classification of lupus nephritis,[29] the Oxford Classification of IgA nephropathy,[47,48] and the Banff classification of allograft pathology. International consensus groups have published recommendations for Pathologic Classification, Diagnosis, and Reporting of GN[49] and for the standardized grading of chronic changes in native kidney biopsy specimens.[50]

The interpretation of renal biopsies requires the pathologist to integrate the biopsy findings with detailed clinical information and therefore requires a thorough understanding of renal disease and the therapeutic implications of the biopsy diagnosis. Close communication between the clinician and pathologist is essential and it is generally very helpful for the biopsy to be viewed and discussed at a clinicopathologic conference so that full discussion of the implications of the biopsy specimen appearances for patient management can take place.

CONCLUSION

Percutaneous renal biopsy is generally safe if care is taken to select and prepare the patients beforehand. It has become a cornerstone of nephrological practice and its handling and interpretation should be made by those experienced in renal pathology. The interpretation of the biopsy should be carried out with adequate clinical information for integrated clinicopathologic conclusions to be drawn.

Complete reference list available at ExpertConsult.com.

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BOARD REVIEW QUESTIONS

1. Relative contraindications to percutaneous renal biopsy include the following:
   a. Uncontrolled hypertension
   b. Bleeding diathesis
   c. Hydronephrosis
   d. Single kidney
   e. Uncooperative patient
   Answer: d
   Rationale: All the others are absolute contraindications (Table 26.2).

2. The best stain for viewing glomerular architecture using light microscopy is:
   a. Hematoxylin and eosin (H&E)
   b. Periodic acid–Schiff (PAS)
   c. Silver methenamine
   d. Masson trichrome
   e. Congo red
   Answer: b
   Rationale: PAS distinguishes the mesangial matrix and glomerular basement membrane from the other components of the glomerulus and therefore allows good assessment of amount of mesangial matrix, mesangial hypercellularity, and the thickness of the basement membrane.

3. Pathology that affects only part of a glomerulus is termed:
   a. Segmental
   b. Mild
   c. Limited
   d. Focal
   e. Sclerosis
   Answer: a
   Rationale: A lesion that affects only part of a glomerulus, that is, with some capillary lumens remaining uninvolved, is called segmental.

4. Electron microscopy is not essential for making a diagnosis of:
   a. Alport syndrome
   b. Thin membrane disease/lesion
   c. Immunoglobulin A nephropathy
   d. Immunotactoid glomerulopathy
   e. Fibrillary glomerulopathy
   Answer: c
   Rationale: See Box 26.5.

5. In a renal biopsy, a lesion containing an acellular structureless material consisting of glycoproteins and sometimes lipids that stains intensely with eosin and periodic acid–Schiff (PAS) but not with silver stains is termed:
   a. Sclerosis
   b. Fibrosis
   c. Necrosis
   d. Amyloid
   e. Hyalinosis
   Answer: e
   Rationale: See Box 26.4.

27

Biomarkers in Acute and Chronic Kidney Diseases

Chirag R. Parikh | Jay L. Koyner

CHAPTER OUTLINE

KEY POINTS

- An ideal biomarker is easily measurable, reproducible, sensitive, organ-specific, cost-effective, easily interpretable, and present in readily available specimens (e.g., blood and urine).
- Due to the inherent variability in the serum creatinine assay, patients with advanced chronic kidney disease (CKD) may be misclassified as acute kidney injury (AKI) based on small changes in creatinine levels.
- Serum cystatin C performs on par with serum creatinine for estimates of glomerular filtration rate (GFR) in the setting of CKD and may provide additional information about a CKD patient's risk for cardiovascular morbidity and mortality.
- Urinary α_1-microglobulin, a low-molecular-weight glycoprotein and member of the lipocalin superfamily, has been associated with the increased risk of CKD and all-cause mortality in a variety of clinical settings.
- Heart-type fatty acid binding protein, proenkephalin, and monocyte chemoattractant protein-1 are promising AKI biomarkers in the setting of cardiac surgery and intensive care unit–associated AKI
- Urinary TIMP-2 × IGFBP-7 is associated with the increased risk of development of Kidney Disease: Improving Global Outcomes stage 2 or 3 AKI in the next 12 hours in ICU patients at high risk for AKI.
- Plasma levels of *TGFR1*, *TGFR2*, EGF, and KIM-1 have been increasingly recognized as potential biomarkers for incident and progressive CKD.

Kidney disease is a global health problem. Acute kidney injury (AKI) and chronic kidney disease (CKD) are increasing in incidence.[1] In the United States, it is clear that the incidence of AKI, regardless of its severity, has been steadily increasing at a rate that is disturbingly high, and it is increasingly recognized that AKI predisposes to the progression of CKD toward end-stage renal disease (ESRD), which ultimately requires dialysis or kidney transplantation.[2–4] According to the World Health Organization, approximately 850,000 patients develop ESRD every year.[5–7] Across the globe, treatment of ESRD poses a major challenge for health care systems and the global economy. The burden of kidney disease is most significant in developing countries and is adversely influenced by inadequate socioeconomic and health care infrastructures.[5,8,9] Importantly, kidney disease progression may be curtailed if the disease is diagnosed early. Hence, detection and management of kidney diseases, whether acute or chronic, in the early, reversible, and potentially treatable stages, is of paramount importance. Biomarkers that will help diagnose kidney injury, predict progression of kidney disease, and provide information regarding the effectiveness of therapeutic intervention will be important adjuncts to our standard management strategies.

Recently, many novel, high-throughput technologies in the fields of genomics, proteomics, and metabolomics have made it easier to interrogate hundreds or even thousands of potential biomarkers at once, without prior knowledge of the underlying biology or pathophysiology of the system

being studied.[10–13] As a result, there is a renewed interest in discovering novel biomarkers for use in drug development and patient care. Despite notable achievements, however, only a few biomarkers—blood urea nitrogen (BUN) level, creatinine concentration, urinalysis results and proteinuria—are routinely used to diagnose and monitor kidney injury. These commonly used gold standard biomarkers of kidney function are not optimal to detect injury or dysfunction early enough to allow prompt therapeutic intervention. Although additional candidate biomarkers have been reported, none have been adequately validated to justify their use in making patient care decisions, but a few look quite promising.

BIOMARKER DEFINITION

In 2001, the U.S. Food and Drug Administration (FDA) standardized the definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic intervention."[14] The National Institutes of Health further classified biomarkers based on their utility (Table 27.1).[14] Biomarkers can potentially serve a wide range of functions in drug development, clinical trials, and therapeutic management strategies. There are many different classes of biomarkers—prognostic, predictive, pharmacodynamic, and surrogate biomarkers. Of note, these categories are not mutually exclusive. Definitions of the different types of biomarkers can be found in Table 27.1. Examples of biomarkers are proteins, lipids, genomic or proteomic patterns, imaging determinations, electrical signals, and cells present in urine. Some biomarkers also serve as surrogate endpoints. A surrogate endpoint is a biomarker intended to substitute for a clinical endpoint. Furthermore, a surrogate endpoint biomarker is expected to predict clinical benefit (harm or lack of benefit) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence.[15] An ideal biomarker is easily measurable, reproducible, sensitive, cost- effective, easily interpretable, and present in readily available specimens (blood and urine).

Table 27.1 Biomarker Definitions

Term	Definition
Biomarker	A characteristic that is objectively measured and evaluated as an indicator of a normal biologic process, pathogenic process, or pharmacologic response to therapeutic intervention. • A prognostic biomarker is a baseline patient or disease characteristic that categorizes patients by degree of risk for disease occurrence or progression, informing about the natural history of the disorder in the absence of a therapeutic intervention. • A predictive biomarker is a baseline characteristic that characterizes patients by their likelihood for response to a particular treatment, predicting either a favorable or unfavorable response. • A pharmacodynamic biomarker is a dynamic assessment that shows that a biologic response has occurred in a patient who has received a therapeutic intervention. Pharmacodynamic biomarkers may be treatment-specific or broadly informative of disease response, with the specific clinical setting determining how the biomarker is used and interpreted.
Clinical endpoint	A characteristic or variable that reflects how a patient fares or functions or how long a patient survives
Surrogate endpoint biomarker (type 2 biomarker)	A marker that is intended to substitute for the clinical endpoint. A surrogate endpoint is expected to predict clinical benefit, harm, lack of benefit, or lack of harm on the basis of epidemiologic, therapeutic, pathophysiologic, or other scientific evidence.

PROCESS OF BIOMARKER DISCOVERY, ASSAY VALIDATION, AND QUALIFICATION IN A CLINICAL CONTEXT

Primary challenges to the development of biomarkers for kidney injury and toxicity are discovery of candidate markers, design of an assay, validation of the assay, and qualification of the biomarker for use in specific clinical contexts. The process of biomarker identification and development is arduous and involves several phases.[16,17] For the purpose of simplicity, this process can be divided into the following five phases (adapted and modified from Pepe and colleagues[16]).

PHASE 1: DISCOVERY OF POTENTIAL BIOMARKERS THROUGH UNBIASED OR HYPOTHESIS-GENERATING EXPLORATORY STUDIES

The primary goal of phase 1 is to identify potential leads using various technologies and to confirm and prioritize the identified leads. The search for biomarkers often begins with preclinical studies that compare either tissue or biologic fluids in diseased animals (e.g., animals with kidney injury) with those in healthy animals to identify genes or proteins that appear to be upregulated or downregulated in diseased tissue relative to control tissue. When biologic samples, such as blood and urine, are readily available from humans, it is possible to forgo the animal model stage. Innovative discovery technologies include microarray-based gene expression profiling that provides information regarding expression of genes, micro RNA (miRNA)-based expression, and proteomic technologies, as well as metabolomic, profiling of biologic fluids based on mass spectrophotometry, and other technologies. The candidate marker approach, especially when informed by the pathophysiology of the disease for which the biomarker is being evaluated, should not be ignored.

Once a promising biomarker is discovered, the validation process begins. An assay has to be developed and validated. The validation process is laborious and expensive, requiring access to patient samples with complete clinical annotation and long-term follow-up, as described in the section on phase 2. In addition, each biomarker must be qualified for

specific application. This is especially true in the case of kidney diseases, for which one biomarker alone may not satisfy the requirements of an ideal biomarker. This is described in the subsequent section on phase 4. Incorporation of several of these novel biomarkers into a biomarker panel may enable simultaneous assessment of site-specific kidney injury or several mechanisms contributing to clinical syndromes.

PHASE 2: DEVELOPMENT AND VALIDATION OF AN ASSAY FOR THE MEASUREMENT OR IDENTIFICATION OF THE BIOMARKER IN CLINICAL SAMPLES

The primary goal of phase 2 is to develop and validate a clinically useful assay that has the ability to distinguish a person with kidney disease or injury from a person with healthy kidneys in a high-throughput fashion. This phase involves development of an assay, optimization of assay performance, and evaluation of the reproducibility of the assay results within and among laboratories. Defining reference ranges of biomarker values is a crucial step before the biomarker can be used clinically.[18,19] It is important to characterize how the levels of these markers vary with patient age, gender, race, and ethnicity, and how biomarker values are related to known risk factors.[20]

PHASE 3: DEMONSTRATION OF THE BIOMARKER'S POTENTIAL CLINICAL UTILITY IN RETROSPECTIVE STUDIES

In phase 3, the primary objectives are to as follows: (1) evaluate the biomarker potential in samples obtained from a completed clinical study; (2) test the diagnostic potential of the biomarker for early detection; and (3) determine the sensitivity and specificity of the biomarker using defined threshold values of the biomarker for utility in prospective studies. For example, if the levels of biomarker differ significantly between cases (those with acute or chronic kidney injury) and control subjects only at the time of clinical diagnosis, then the biomarker shows little promise for population screening or early detection. In contrast, if levels differ significantly at hours, days, or years before clinical symptoms appear, then the biomarker's potential for early detection is increased. This phase also involves comparing the biomarker with several other novel biomarkers or existing gold standard biomarkers and defining the biomarker's performance characteristics (i.e., sensitivity, specificity) using receiver-operating characteristic curve analysis. This latter process is particularly challenging in kidney disease, given uncertainties in the sensitivity and specificity of the gold standard used.[21]

PHASE 4: PERFORMANCE OF PROSPECTIVE SCREENING STUDIES

The primary aim of phase 4 studies is to determine the operating characteristics of the biomarker in a relevant population by measuring detection and false referral rates. In contrast to phase 1, 2, and 3 studies, which are based primarily on stored specimens, studies in phase 4 involve screening subjects prospectively and demonstrating that clinical care is changed as a result of the information provided by the biomarker analysis.

BIOMARKER QUALIFICATION PROCESS

The application for FDA qualification of novel biomarkers requires the intended use of the biomarker in nonclinical and clinical contexts and the collection of evidence supporting qualification. This can be a joint and collaborative effort among regulatory agencies, pharmaceutical companies, and academic scientists.[22]

Data are shared between the FDA and pharmaceutical industry or academic laboratories through voluntary exploratory data submissions (VXDSs).[23] Submission of exploratory biomarker data through VXDSs allows interaction between reviewers at the FDA and researchers in industry or academia regarding study designs, sample collection and storage protocols, technology platforms, and data analysis. This pilot process for biomarker qualification allows the Predictive Safety Testing Consortium to apply to both U.S. and European drug authorities simultaneously for qualification of new nephrotoxic biomarkers (e.g., kidney injury molecule-1, albumin, total protein, cystatin C, clusterin, trefoil factor 3, and α_2-microglobulin) as predictors of drug-mediated nephrotoxicity.[23–25] The FDA and the corresponding European authority (European Medicines Agency [EMA]) reviewed the application separately and made decisions as to whether each would allow the new biomarkers to be "fit for purpose" in preclinical research.[24,25] Some of these markers were proposed to be qualified as biomarkers for clinical drug-induced nephrotoxicity once further supportive human data are submitted. More recently, the FDA has approved total kidney volume as a biomarker of disease progression for the purposes of clinical trial enrichment in autosomal dominant polycystic kidney disease (see also Chapter 45), thus opening to door to other novel measures as potential biomarkers of disease progression in the setting of acute kidney injury (AKI) and other forms of chronic kidney disease (CKD).[22,26]

It is notable that the process described above is specific for the FDA and United States and that the biomarker validation and approval process varies significantly around the world. In the past few years, the FDA, EMA, and other agencies have approved several biomarkers for clinical use in the United States and other countries throughout Europe and Asia.

PHASE 5: CONTINUED ASSESSMENT OF THE VALIDITY OF THE BIOMARKER IN ROUTINE CLINICAL PRACTICE

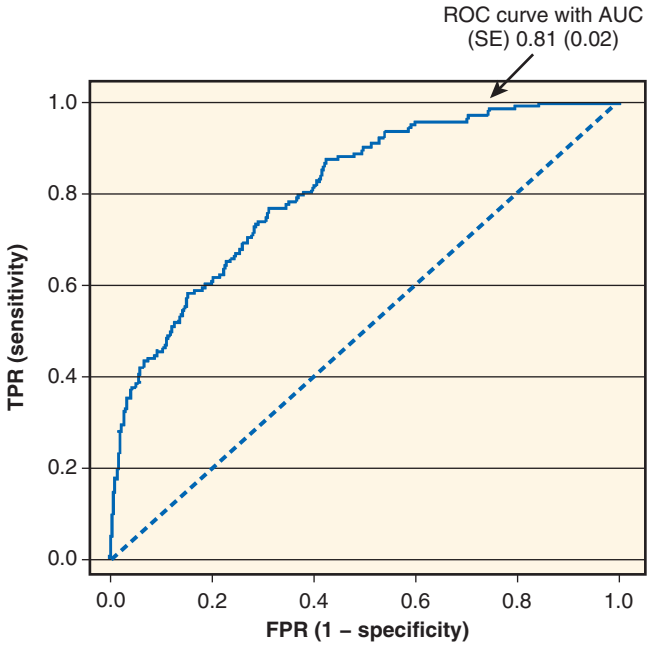
Phase 5 addresses whether measurement of the biomarker alters physician decision making and/or reduces the mortality or morbidity associated with the given disease in the population.

ANALYSIS OF BIOMARKER PERFORMANCE

The widely accepted measure of biomarker sensitivity and specificity is the receiver-operating characteristic (ROC) curve.[27] ROC curves display the proportion of subjects, with and without disease, correctly identified at various cutoff points. A ROC curve is a graphic display of trade-offs between the true-positive rate (sensitivity) and false-positive rate (1 – specificity, where specificity is expressed as a value

from 0 to 1) when the biomarker is a continuous variable (Fig. 27.1).[28,29] Sensitivity is plotted along the ordinate, and the value of (1 – specificity) is plotted on the abscissa. Each point on the curve represents the true-positive rate and false-positive rate associated with a particular test value. The diagonal, represented by the equation true-positive rate (sensitivity) = false-positive rate (1 – specificity), corresponds to the set of points for which there is no selectivity in predicting disease. The area under this line of "unity" is 0.5, which indicates no advantage relative to the flip of a coin.

The performance of a biomarker can be quantified by calculating the area under the ROC curve (AUC). The AUC is the probability that a randomly sampled case has a larger biomarker value (or risk score) than a randomly sampled control. Although this makes the AUC easily interpretable, this interpretation is not always clinically meaningful, because cases and controls do not present to clinicians in random pairs. Thus, whereas an ideal biomarker could supply an AUC of 1.0 (a clinical rarity), in actuality the AUC lacks true direct clinical relevance.[29] Despite these flaws, the AUC is widely reported and familiar to clinicians. The shortcomings of AUC extend into the assessment of the incremental change in AUC (ΔAUC) when adding a new marker to a group of previously established predictors. The clinical impact of a ΔAUC of 0.02 is often unclear, and the statistics and *P* values behind such calculations remain problematic.[30,31]



		True disease state	
		Diseased	**Nondiseased**
Biomarker test	Positive (diseased)	True-positive (TP)	False-positive (FP)
	Negative (nondisease)	False-negative (FN)	True-negative (TN)

Biomarker classification by disease status
True-positive rate (TPR) = sensitivity = TP/(TP + FN)
False-positive rate (FPR) = 1 – specifity = FP/(FP + TN)

Fig. 27.1 Receiver operator characteristic curves.

Other important parameters related to biomarker performance, primarily with respect to the testing of larger or specific populations, are positive and negative predictive values. The positive predictive value is the proportion of persons who test positive for a disease and actually have the disease, whereas the negative predictive value represents the proportion of persons who test negative and do not have the disease. There is considerable interest in developing algorithms that use a composite of values of several biomarkers that are measured in parallel for the purpose of increasing diagnostic potential or predicting disease course and patient outcomes.

More recently, the Net Reclassification Index (NRI) and the Integrated Discrimination Improvement Indices (IDIs) have been used to evaluate the ability of new biomarkers. The NRI is simply the proportion of the population whose risk category changes with the new biomarker; a small reclassification rate means that treatment decisions will rarely be altered by the new biomarker. IDI is defined as the difference in discrimination slopes between the unadjusted and biomarker-adjusted clinic models, with large effect sizes having an IDI ≥ 0.10 and medium effect sizes having an IDI between 0.05 and 0.10.[32] Note that the NRI and IDI have not been widely accepted by all statisticians.[33]

CHARACTERISTICS OF AN IDEAL BIOMARKER FOR KIDNEY DISEASE

Characteristics of an ideal biomarker for kidney disease are described in Table 27.2. For AKI, the biomarker should have the following characteristics: (1) be organ-specific and allow differentiation between intrarenal, prerenal, and postrenal causes of AKI, as well as acute glomerular injury; (2) be able to detect AKI early in the course and predict its course and potentially the future implications of AKI; (3) be able to identify the cause of AKI; (4) be site-specific and able to inform pathologic changes in various segments of renal tubules during AKI, as well as correlate with the histologic findings in kidney biopsy specimens; (5) be easily and reliably measured in a noninvasive or minimally invasive manner; (6) be stable in its matrix; (7) be rapidly and reliably measurable at the bedside; and (8) be inexpensive to measure.

In CKD (unlike AKI), the timing and nature of the insult are very hard to estimate, which makes the search for early biomarkers for CKD very difficult. An ideal biomarker for CKD shares many of the same requirements described earlier for AKI biomarkers, including providing insight into the following: (1) the location of the injury (e.g., glomerular, interstitial, tubular); (2) the disease mechanism; (3) the progressive course of the disease; and (4) the risk of complications from comorbid conditions such as cardiovascular disease and diabetes.

ACUTE KIDNEY INJURY MARKERS

In the cardiac sciences, the discovery of biomarkers, such as troponins, which reflect early cardiomyocyte damage rather than decreased cardiac function, has enabled the development and implementation of novel therapeutic strategies to reduce coronary insufficiency and associated morbidity and mortality.[34,35] By contrast, the delay in diagnosis associated

Table 27.2 Characteristics of an Ideal Kidney Biomarker

Functional Properties	Physiochemical Properties
• Rapid and reliable increase in response to kidney diseases • Highly sensitive and specific for acute and/or chronic kidney disease • Shows good correlation with degree of renal injury • Provides risk stratification and prognostic information (e.g., severity of kidney disease, need for dialysis, length of hospital stay, and mortality) • Site-specific to detect early injury (e.g., proximal, distal, interstitium or vasculature) and identify pathologic changes in specific segments of renal tubules • Applicable across different races and age groups • Allows recognition of the cause of kidney injury or disease (e.g., ischemia, toxins, sepsis, cardiovascular disease, diabetic nephropathy, lupus, or combinations) • Organ-specific; allows differentiation among intrarenal, prerenal, and extrarenal causes of kidney injury • Noninvasively identifies the duration of kidney failure (acute kidney injury, chronic kidney injury) • Useful to monitor the response to therapeutic interventions • Provides information on the risk of complications from comorbid conditions (especially in chronic kidney disease)	• Stable over time across different temperature and pH conditions, with clinically relevant storage conditions • Rapidly and easily measurable • Not subject to interference by drugs and endogenous substances

Table 27.3 Kidney Disease: Improving Global Outcomes (KDIGO) Staging of Acute Kidney Disease

Stage	Serum Creatinine Criteria	Urine Output Criteria
1	1.5–1.9 times baseline *or* ≥0.3 mg/dL (26.5 μmol/L) increase	<0.5 mL/kg for 6–12 h
2	2.0–2.9 time baseline	<0.5 mL/kg/h for ≥12 h
3	≥3.0 times *or* Increase in serum creatinine to ≥4.0 mg/dL (≥353.6 μmol/L) *or* Initiation of renal replacement therapy *or* In patients <18 years, decrease in eGFR to <35 mL/min^2	<0.3 mL/kg/h for ≥24 h or anuria for 12 h

with the use of kidney biomarkers, such as serum creatinine concentration, has impaired the ability of nephrologists to conduct interventional studies in which the intervention can be implemented early in the course of the disease process.[36] Although the past decade has seen a revolution in terms of diagnostic criteria for AKI with the RIFLE (*r*isk, *i*njury, *f*ailure, *l*oss, *e*nd-stage kidney disease) classification[37] and the Acute Kidney Injury Network (AKIN) definition of AKI[38] being harmonized into the Kidney Disease: Improving Global Outcomes (KDIGO) classification[39] (Table 27.3), these criteria remain limited by their reliance on the serum creatinine concentration on some level. More recently, there has been a call to expand these definitions further to potentially include biomarkers, but as of the time of this publication, these new guidelines have yet to be widely accepted.[40] These new guidelines and the concept of AKI remain reliant on the serum creatinine level and will continue to serve as a limitation, given creatinine's role as a functional biomarker. The serum creatinine level can increase in cases of prerenal azotemia when there is no tubular injury and can be unchanged under conditions of significant tubular injury, particularly when patients have good underlying kidney function and significant kidney reserve. Nonetheless, these criteria have advanced our understanding of the epidemiology of AKI, and these standardized consensus definitions have allowed for comparisons and aggregation of data from a larger number of papers.[41] Biomarkers of AKI can serve several purposes and are no longer thought of as a replacement for the serum creatinine level. Table 27.4 summarizes several of the potential uses of AKI biomarkers. Fig. 27.2 summarizes the kidney-specific location of the AKI biomarkers discussed later.

Urine and serum biomarkers each have advantages and disadvantages. Serum biomarkers are often not stable and are difficult to measure because of interference with several serum proteins. By contrast, urinary biomarkers are relatively stable and easy to assess; however, their concentrations are greatly influenced by the hydration and volume status of the patient and other conditions that affect urinary volume. To overcome this challenge, urinary biomarker concentrations have often been normalized to urinary creatinine concentrations to eliminate the influence of urinary volume on the assumption that the urinary creatinine excretion rate is constant over time, and that biomarker production or excretion has a linear relationship with the urinary creatinine excretion rate. Bonventre and colleagues have challenged this assumption, especially in AKI settings, when the urine creatinine excretion rate is not constant and changes over time, greatly influencing the normalized value of a putative urinary biomarker after normalization. They have suggested that the most accurate method to quantify biomarkers is the timed collection of urine samples to estimate the renal excretion rate[42]; however, this approach is not practical for

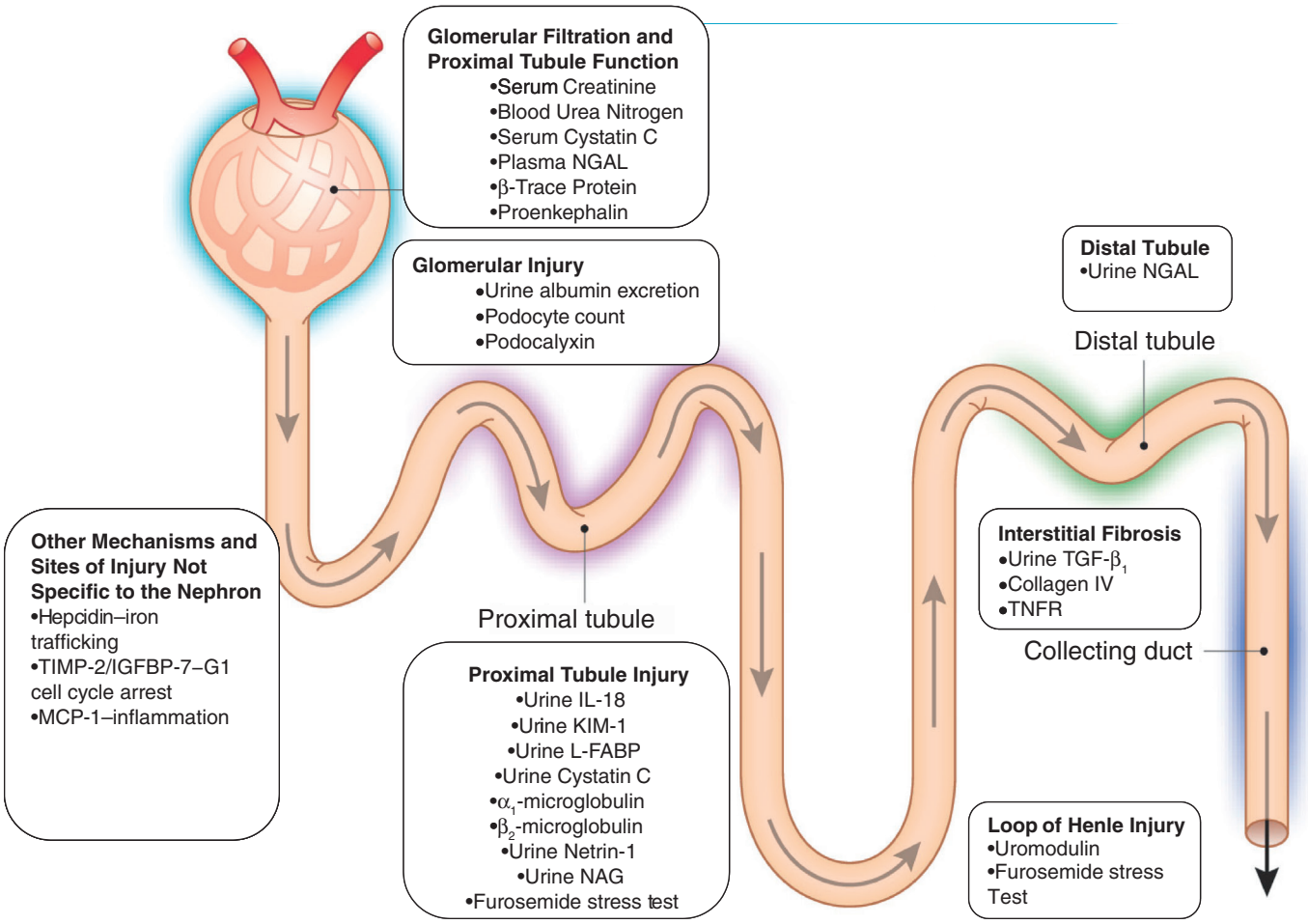


Fig. 27.2 Biomarkers in relation to their site of injury in the nephron. *GST,* Glutathione S-transferase; *IGFBP7,* insulin-like growth factor binding protein-7; *IL-18,* interleukin-18; KIM-1, kidney injury molecule-1; *L-FABP,* liver-type fatty acid binding protein; *MCP-1,* monocyte chemotactic protein 1; *NAG,* N-acetyl-β-D-glucosaminidase; *NGAL,* neutrophil gelatinase-associated lipocalin; *TGF-β_1,* transforming growth factor-β_1; *TIMP-2,* tissue inhibitor metalloproteinase-2; *TNFR,* tumor necrosis factor receptor. (Adapted from Koyner JL, Parikh CR. Clinical utility of biomarkers of AKI in cardiac surgery and critical illness. *Clin J Am Soc Nephrol.* 2013;8:1034–1042.)

Table 27.4 Potential Uses for Biomarkers of Acute Kidney Injury (AKI) and Chronic Kidney Disease (CKD)

Disorder	Potential Use
AKI	Early detection of AKI: • Differential diagnosis of AKI (e.g., distinguishing between volume-mediated AKI [prerenal] and intrinsic tubular injury (e.g., acute tubular necrosis) • Predicting outcomes of AKI at the time of clinical diagnosis (need for RRT, development of post-AKI CKD, short- and long-term mortality) • Predicting recovery from AKI • Ascertaining the nephron-specific location and cause of renal injury • Monitoring the effects of an intervention
CKD	Early detection and diagnosis of CKD: • Predicting the progression of CKD (rapid vs. slow progression) • Predicting outcomes of CKD at the time of clinical diagnosis (development of ESRD, short- and long-term mortality) • Predicting cardiovascular disease and outcomes in patients with CKD • Monitoring the effects of an intervention

routine clinical care. Endre and colleagues delved into this issue further by demonstrating that the ideal method for quantitating biomarkers of urinary AKI depends on the outcome of interest; absolute biomarker concentrations best diagnosed AKI at the time of intensive care unit (ICU) admission, whereas normalization to urinary creatinine improved the prediction of incipient AKI.[43] A potential explanation of the failings of normalization is that it will often amplify the signal. For example, when the glomerular filtration rate (GFR) is reduced in immediate response to a tubular injury, the amount of biomarker produced will increase, and the urinary creatinine level will decrease. The normalized value will therefore increase by a greater amount in the short term than can be explained by the increase in the absolute level of biomarker production. Currently, there is no standardized method of accounting for this issue, with some urinary biomarkers being normalized to urine creatinine and others being reported without normalization.

Because AKI and CKD share functional and structural aspects, there are overlapping as well as distinct classes of functional and structural biomarkers. Among the functional markers, the GFR is often used as the gold standard. Although the true GFR, as determined by agents that are freely filtered and undergo minimal handling by the tubule (e.g.,

Table 27.5 Biomarker Performance in Detecting Acute Kidney Injury (AKI)[a]

	Perioperative AKI				Critically Ill			Emergency Room	
Parameter	Preop AKI Risk	Early Postop AKI	AKI Progression	Long-Term Mortality	Early Diagnosis of AKI	Type of AKI (Transient vs. Intrinsic)	Need for RRT	Early Diagnosis of AKI	Type of AKI (Transient vs. Intrinsic)
Urine NGAL	N/A	+	–	+	+	+	+	+	+
Blood NGAL	–	+	+	+	–	?	–	?	?
Blood CysC	+	+	–	?	+	+	+	?	?
Urine CysC	N/A	–	–	–	+	+	+	+	+
Urine IL-18	N/A	+	+	+	+	+	+	+	+
Urine KIM-1	N/A	+	–	+	+	–	–	+	+
Urine L-FABP	N/A	–	–	+	?	?	–	+	+
TIMP-2 IGFBP-7	N/A	+	+	?	+	?	+	+	?
Urine protein, albumin	+	+	+	+	?	?	?	?	?

[a]From multicenter studies at a variety of clinical time points.

CysC, Cystatin C; *IL-18,* interleukin-18; *KIM-1,* kidney injury molecule-1; *L-FABP,* liver fatty acid binding protein; *NGAL*, neutrophil gelatinase-associated lipocalin; *Preop,* preoperative; *Postop,* postoperative; *RRT,* renal replacement therapy; *TIMP-2, IGFBP-7,* tissue inhibitor metalloprotease 2, insulin-like growth factor binding protein-7.

+, Data published display the ability to detect this aspect of AKI; –, data published do not display the ability to detect this aspect of AKI; *?,* no large multicenter data published on this biomarker or aspect of AKI; *N/A,* Not applicable to the following: (1) biomarkers of tubular injury have no role in preoperative risk screening; (2) serum creatinine is intrinsic to the definitions of AKI being tested.

Adapted and expanded from Koyner JL, Parikh CR. Clinical utility of biomarkers of AKI in cardiac surgery and critical illness. *Clin J Am Soc Nephrol.* 2013;8:1034–1042.

iothalamate, iohexol, inulin), represents a sensitive measure for determining changes in kidney function, these tests are invasive and laborious to perform. Moreover, because of the renal reserve, changes in GFR may not indicate structural injury until significant injury has occurred. On the other hand, structural markers of tubular injury are expressed by tubular cells, and subtle changes in epithelial cells lead to release of these markers into the urine. It is becoming increasingly clear that many of these biomarkers serve as signals for both AKI and CKD and also may be used to monitor progression from AKI to CKD. A challenge is to define at what level of release of these markers the injury is clinically significant in either the acute or chronic setting. Failure to identify the separate impacts of CKD and AKI on these biomarker values will lead to inappropriate clinical decision and/or poor results in clinical studies.[44,45]

Table 27.5 summarizes the ability of biomarkers to detect clinical endpoints related to AKI in a variety of clinical settings.

GLOMERULAR INJURY MARKERS

SERUM GLOMERULAR FILTRATION MARKERS

During the course of injury, kidney function may be impaired with reduction in the GFR and accumulation of several nitrogenous compounds in the blood. Serum creatinine and BUN concentrations are routinely used as markers of kidney injury, but it is important to recognize these parameters as markers for kidney dysfunction, rather than direct markers of injury.

As discussed elsewhere in this text, the estimated GFR (eGFR), using creatinine as a biomarker, is most reliable for CKD under steady-state conditions. In the acute setting, its use is more problematic for reasons that have already been discussed. In healthy persons, the GFR is in the range of 90 to 130 mL/min/1.73 m^2. By definition, patients with stage 4 or 5 CKD have GFRs that are below 30 mL/min/1.73 m^2.[46] Complications of CKD are more pronounced at lower GFRs, and mild to moderate CKD may progress to end-stage renal disease (ESRD).

In AKI, the GFR is only indirectly linked to kidney injury, and changes in the GFR reflect a late consequence in a sequence of events associated with a primary insult to the kidney. Furthermore, because of renal reserve, a large amount of functioning renal tissue can be lost without significant changes in the GFR.[47,48] The functional effects of renal reserve on the GFR can be demonstrated in kidney donors, who often have only modest changes in serum creatinine levels and GFR after donating one kidney, even though 50% of the renal mass is lost.[49]

Ideally, a serum GFR marker should be freely filtered, with no reabsorption or secretion in the tubule, and should maintain a constant plasma level when kidney function is stable. GFR can be determined using exogenous and endogenous markers of filtration. Evaluation of the GFR using the exogenous markers inulin, iothalamate, or iohexol provides reliable results and represents the gold standard; however, the process is time-consuming and expensive and can be performed only in specialized settings.[46] Once the GFR level falls below 60 mL/min/1.73 m^2, renal functional impairment can be estimated adequately by the serum creatinine level using various equations to calculate the eGFR.[50–52] Although traditionally these equations have been less accurate for those with higher GFRs, newer formulas have been constructed using more patients with normal and near-normal GFRs.[52]

Creatinine

Determination of the eGFR using endogenous creatinine is cost-effective but can be problematic. Creatinine is a

breakdown product of creatine and phosphocreatine, which are involved in the energy metabolism of skeletal muscle. Creatinine is freely filtered by the glomerulus, but is also to a lesser degree (10%–30%) secreted by the proximal tubule. Under normal conditions, the daily synthesis of creatinine of approximately 20 mg/kg of body weight reflects muscle mass and varies little.[53]

Accumulated data from various studies have indicated that the creatinine concentration is not an ideal marker for diagnosing AKI for a variety of reasons, including the following[54–56]:

1. Creatinine production and its release into the circulation vary greatly with age, gender, muscle mass, certain disease states and, to a lesser extent, diet. For example, in rhabdomyolysis, serum creatinine concentrations may rise more rapidly due to the release of preformed creatinine from the damaged muscle. Also, body creatinine production, as measured by 24-hour urinary excretion, decreases with older age, falling from a mean of 23.8 mg/kg of body weight in men aged 20 to 29 years to 9.8 mg/kg of body weight in men aged 90 to 99 years, largely because of the reduction in muscle mass.[57]
2. Serum creatinine concentrations are not specific for renal tubular injury. For example, intravascular volume depletion "prerenal" factors (e.g., severe dehydration, blood volume loss, altered vasomotor tone, age-related decrease in renal blood flow) and postrenal factors (e.g., obstruction or extravasation of urine into the peritoneal cavity) may falsely elevate serum concentrations in the absence of parenchymal damage. Thus, a decrease in the eGFR inferred from an increase in the serum creatinine level may not distinguish among prerenal, intrinsic renal, and postrenal causes of impaired kidney function, which may not be the case for some biomarkers of renal tubular injury.[58] Even in cases in which the serum creatinine level is elevated as a consequence of direct renal injury, it cannot be used to determine the location of the injury (glomerular vs. tubular, or proximal tubular vs. distal tubular).
3. Static measurement of the serum creatinine level does not reflect the real-time changes in the GFR resulting from acute changes in kidney function as creatinine accumulates over time. Given the large amounts of functional kidney reserve in healthy persons and the variable amounts of kidney reserve in patients with mild to moderate disease, the creatinine level is not a sensitive marker.[59]
4. Drug-induced reduction in tubular secretion of creatinine might result in underestimation of kidney function. Medications such as cimetidine and trimethoprim inhibit creatinine secretion and increase the serum creatinine concentration without affecting the true GFR.[60,61]
5. One commonly used creatinine assay—the Jaffe method—is subject to interference by intake of certain drugs or by certain pathophysiologic states, including hyperbilirubinemia and diabetic ketoacidosis.[60]
6. Small changes in serum creatinine levels are subject to high false-positive rates, and this effect is magnified in those with a higher baseline creatinine due to the inherent variability in the creatinine assay. Thus patients with CKD may be misclassified as having AKI based solely on small changes in the serum creatinine level.[62]

Similarly, the use of serum creatinine levels in CKD is limited by several patient-dependent and independent variables, including age, race, sex, and comorbid conditions. Serum creatinine concentration can significantly decrease in advanced kidney disease, unrelated to its renal clearance.[63] The sensitivity of serum creatinine levels in determining kidney function can be improved by serial measurements of timed creatinine clearance (usually, but not always, 24-hour collections). However, many individuals find this collection cumbersome, and errors (e.g., skipped voids) typically lead to underestimation of function.

The serum level creatinine is stable during long-term storage, after repeated thawing and refreezing,[64] and for up to 24 hours in clotted whole blood at room temperature.[65] The Jaffe reaction–based assay (alkaline picrate assay) is routinely used in clinical laboratories to assess creatinine levels. However, Jaffe methods overestimate serum creatinine concentration by approximately 25% due to the interference of noncreatinine chromogens, particularly proteins. Interference from glucose[66,67] and acetoacetate[68] are particularly important because diabetic patients are particularly prone to develop CKD. As a result, eGFRs are higher when Jaffe methods are used than when other approaches are used. Expert professional bodies have recommended that all methods of creatinine measurement should become traceable to a reference method based on isotope dilution mass spectrometry.[69] Several modifications of the Jaffe method have been made to increase the specificity by decreasing the influence of interfering substances.[70,71] Enzymatic methods of measuring creatinine have been widely adopted by clinical laboratories as an alternative to alkaline picrate assays. Although various substances do interfere with enzymatic assays, the assays are reported to be subject to less interference than Jaffe methods.[72–74] The high-performance liquid chromatography (HPLC)–based assay has evolved as a potential alternative approach for measurement of the serum creatinine level.[75,76] Several studies have demonstrated that HPLC methods have greater analytic specificity than conventional methods.[77–79] This approach clearly has severe limitations with respect to throughput, however.

Finally, over the past decade, there has been a dedicated national effort (within the United States) to standardize serum creatinine assays by establishing calibration traceability to an isotope dilution mass spectrometry (IDMS) reference standard. Prior to standardization, there was a large variability in serum creatinine results among clinical laboratories, with roughly a 10% to 20% bias being reported in the literature.[80] This process, which started in 2005 and has recently been completed, has led to the standardization of assays, which has led to less variation and more accurate eGFR measurements when used in conjunction with the IDMS-traceable estimating equations.[81,82]

Blood Urea Nitrogen

Blood urea is a low-molecular-weight waste product derived from dietary protein catabolism and tissue protein turnover, and its levels are inversely correlated with decline in the GFR.[83] Urea is filtered freely, and a variable amount (≈30%–70%) is reabsorbed predominantly in the proximal tubule, with recycling between the tubule and interstitium in the kidney medulla. The normal range of urea nitrogen in blood or serum is 5 to 20 mg/dL (1.8–7.2 mmol urea/L).[83]

The wide reference range reflects the influence on BUN of nonrenal factors, including dietary protein intake, endogenous protein catabolism, fluid intake, and hepatic urea synthesis.[83,84] BUN concentrations also increase with excessive tissue catabolism, especially in cases of fever, severe burns, trauma, high corticosteroid dosage, chronic liver disease, and sepsis.[83] In addition, any factor that increases the tubular reabsorption of urea, including decreased effective arterial volume (i.e., impaired renal perfusion) and/or obstruction of urinary drainage, will increase the BUN concentration.[83,85,86] Because of these limitations, BUN is not a sensitive and specific marker for acute or chronic kidney disease. However, for those with advanced CKD (e.g., CKD 4–5), some have suggested averaging urea clearance and creatinine clearance to serve as a more accurate estimate of the true GFR. This is in part because at these lower levels of renal function, creatinine clearance will overestimate the (secretion) GFR, whereas urea clearance will underestimate the GFR.[87] BUN is measured by spectrophotometry. Because of these undesirable limitations of creatinine and BUN as markers, there has been a great deal of interest in the identification of improved biomarkers for kidney injury.

Cystatin C

For the past 10 to 15 years, there has been a tremendous amount of research investigating serum cystatin C as a marker of GFR, and urinary cystatin C excretion has been proposed as a tubular injury marker. In 1961, Butler and Flynn studied the urine proteins of 223 individuals by starch gel electrophoresis and found a new urine protein fraction in the post–gamma globulin fraction.[88] They named this protein fraction "cystatin C." Cystatin C is a low-molecular-weight protein produced at a constant rate by all nucleated cells and eliminated exclusively by glomerular filtration. It has a small size (13 kDa) and a positive charge at physiologic pH. It is neither secreted nor reabsorbed by renal tubules but undergoes almost complete catabolism by proximal tubular cells; thus little, if any, appears in the urine under normal circumstances. Any impairment of reabsorption in proximal tubules can lead to marked increases in urinary levels of cystatin C in humans and animals. There have been a number of studies on the diagnostic potential of both serum and urinary cystatin C levels in acute and chronic kidney disease in humans.

Chronic Kidney Disease. Because of its short half-life (≈2 hours) and other properties described earlier, some believe that serum cystatin C levels reflect the GFR better than creatinine concentration. Initially, it was thought that the serum levels of cystatin C would be unaffected by gender, age, race, and muscle mass but, over the past several years, multiple studies have demonstrated that these factors are associated with altered levels of the biomarker.[89,90] Notably, cystatin C levels have been shown to be associated with factors similar to those associated with creatinine—namely, that these levels may be elevated in males, taller and heavier patients, and those with higher lean body mass.[89–91] However, unlike the serum creatinine level, which is usually lower in older adults, given their decreased muscle mass, a study investigating a subset of over 7500 subjects from the National Health and Nutritional Examination Survey (NHANES) III has demonstrated that cystatin C levels are elevated in more than 50% of those older than 80 years.[91]

Despite these minor limitations, cystatin C remains an excellent biomarker of CKD and performs on par, if not better than, the serum creatinine level in some cases. Equations for estimating GFR and CKD classification are discussed elsewhere in this text (Chapter 23). In a prospective cohort study of 26,643 Americans enrolled in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study, Peralta and colleagues have demonstrated that the cystatin C–based eGFR improves CKD classification and definition, as well risk stratification (for the development of ESRD and death) relative to a creatinine-based eGFR.[92] This correlation with mortality was not novel because cystatin C demonstrated a stronger risk relationship with mortality than creatinine concentration or eGFR in older adults with cardiovascular disease.[93] In the Cardiovascular Health Study cohort of 4637 community-dwelling older adults, higher serum cystatin C concentrations were associated with a significantly elevated risk of death from cardiovascular causes (hazard ratio [HR], 2.27 [1.73–2.97]), myocardial infarction (HR, 1.48 [1.08–2.02]), and stroke (HR, 1.47 [1.09–1.96]) after multivariate adjustment. In the same study, higher serum creatinine values were not independently associated with any of these three outcomes.[94] Furthermore, a study in the general population has suggested that the cystatin C level has a stronger association with cardiovascular disease outcomes than creatinine concentration or eGFR, especially among older adults.[94–96] On the other hand, a study based in primary care has reported that the use of cystatin C to estimate GFR in a predominantly older population with relatively mild CKD resulted in reclassification of a small proportion (7.7%) of participants with CKD category G3aA1, defined by creatinine to GFR (>60 mL/min/1.73 m^2), but a much greater proportion (59%) were reclassified to a more advanced CKD category without any improvement in risk prediction.[97] Thus, serum cystatin C levels may be a better marker of kidney function than serum creatinine concentration in some, but not all, persons.

In addition to older adults, cystatin C has proven superior to serum creatinine in those infected with HIV. Choi and colleagues have demonstrated that the cystatin C–based eGFR outperformed serum creatinine in the ability to predict 5-year all-cause mortality in a cohort of 922 HIV-infected individuals.[98] This study mirrors recent findings from a study of 908 HIV-infected women, which demonstrated that CKD risk factors are associated with an overestimation of GFR by the serum creatinine level relative to cystatin C and that cystatin C significantly improves mortality risk prediction when added to a clinical model that already includes the serum creatinine level.[99]

The concept of using cystatin C in concert, rather than in place of, serum creatinine has been gaining momentum.[100] Using a cross-sectional analyses and data from 5352 participants from 13 previously published studies, Inker and colleagues developed estimation equations using cystatin C alone and cystatin C and creatinine combined.[100] They then went on to validate these equations in a cohort of 1119 participants from 5 different studies. They demonstrated that combined equations outperformed the creatinine-alone equations and, in some cases, led to a NRI of 0.194 ($P < .001$). Although this study was performed predominantly in white subjects, thus limiting its broad applicability, it demonstrates the potential of cystatin C (and other biomarkers) to be used to augment the diagnostic scope of the serum creatinine

Box 27.1 Cystatin C

1. Serum cystatin C performs on par, if not, better than serum creatinine for the identification of patients with chronic kidney disease.
2. Serum cystatin C has been specifically shown to outperform serum creatinine in some older adults as well as those with HIV.
3. Additionally, serum cystatin C outperforms serum creatinine for its ability to detect those at risk for cardiovascular morbidity and mortality.
4. Serum cystatin C should be incorporated into the management and care of patients with chronic kidney disease.

level rather than replace it. Subsequently, the combined creatinine and cystatin C Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation has been shown to outperform each individual equation (creatinine and cystatin C) in a cohort of 805 older community-based individuals.[101] Given the mounting clinical evidence and the emergence of automated assays and cheaper reagent costs (e.g., $4), it is increasingly apparent that cystatin C should become a routine part of the nephrologist laboratory assessment of CKD and points to an increased role for cystatin C in the management of patients with CKD. However, as with any biomarker, its limitations should be considered when interpreting results (Box 27.1).[102]

Acute Kidney Injury. Given its success as a marker of glomerular filtration, several groups have investigated serum cystatin C as a potential biomarker of AKI. In a single-center mixed ICU population of 85 subjects (44 of whom developed RIFLE Classified AKI), Herget-Rosenthal and colleagues demonstrated that serum cystatin C had excellent diagnostic value, predicting AKI 24 and 48 hours prior to serum creatinine (AUC of 0.97 and 082, respectively).[103] These data were followed up by a study of 442 patients from 2 separate ICUs that demonstrated that plasma cystatin C increased earlier than serum creatinine and was able to significantly predict several adverse patient outcomes, including sustained AKI, death, and dialysis.[104] Similarly, in a study of 202 diverse intensive care unit (ICU) patients, of whom 49 developed AKI based on urine output and/or serum creatinine RIFLE F criteria, serum cystatin C levels showed excellent predictive value for AKI. However, the serum cystatin C concentration did not rise earlier than the serum creatinine concentration.[105]

Outside the ICU, cystatin C levels were shown to be capable of detecting a decrease in the GFR after contrast agent administration earlier than the serum creatinine value in adult patients who underwent coronary angiography.[106] In a prospective study of 87 patients who underwent elective catheterization, contrast medium–induced nephropathy occurred in 18 patients, and ROC analysis showed a higher AUC for cystatin C level than for serum creatinine concentration (0.933 vs. 0.832; $P = .012$). When a cutoff value of more than 1.2 mg/L was used, cystatin C level before catheterization exhibited 94.7% sensitivity and 84.8% specificity for predicting contrast medium–induced nephropathy.[107]

Serum cystatin C has been studied as a biomarker for both early AKI (rising earlier than serum creatinine) and AKI severity, with several smaller studies providing mixed results.[108–111] More recently, the larger multicenter Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) study investigated several aspects of serum cystatin C following both adult and pediatric cardiac surgery. In 1147 adults, Shlipak and colleagues demonstrated that preoperative serum cystatin C values outperformed serum creatinine– and creatinine-based eGFRs in its ability to forecast postoperative AKI.[112] After adjustment for clinical variables known to contribute to AKI, serum cystatin C had a C-statistic of 0.70 and an NRI of 0.21 compared with serum creatinine ($P < .001$). However, when this same group investigated sensitivity and rapidity of AKI detection (defined as a 25%, 50%, and 100% increase from preoperative values) by postoperative changes in serum cystatin C, they did not demonstrate a clear advantage over changes in the serum creatinine level.[113] In a follow-up analysis, they demonstrated that postoperative elevations of cystatin C (>25%) were associated with an increased risk of death during a 3-year follow-up period. This long-term mortality risk was higher in those with changes in cystatin C alone (adjusted HR, 2.2 [1.09–4.47] compared with those with changes in just the serum creatinine level (HR, 1.50 [0.96–2.34]).[114] This mixed message of postoperative serum cystatin C in adults is in stark contrast to the results in 288 children undergoing cardiac surgery. Zappitelli and colleagues have demonstrated that serum cystatin C measured within the first 6 postoperative hours is associated with both stages 1 and 2 pediatric AKI.[115] Additionally, postoperative serum cystatin C values were also associated with adverse patient outcomes, including length of mechanical ventilation and length of ICU stay. However unlike the adult population, preoperative values were not associated postoperative AKI.

β-Trace Protein

β-Trace protein (BTP), also referred as "prostaglandin D synthase," has emerged as another promising biomarker for the GFR. BTP is a small protein with a molecular weight of 23 to 29 kDa, depending on the size of the glycosyl moiety. BTP belongs to the lipocalin protein family, whose members are primarily involved in the binding and transport of small hydrophobic ligands. It is primarily produced in the cerebral fluid, where its concentrations are more than 40-fold higher than in the serum. BTP is primarily eliminated by glomerular filtration, and its concentrations in urine range from 600 to 1200 μg/L.[116]

The first observation of elevated BTP levels in association with impaired kidney function was reported by Hoffmann and associates in 1997.[117] Since then, several research studies have been conducted to evaluate the sensitivity and specificity of BTP as a marker of GFR and to compare it with the serum creatinine level in patients with CKD[118] and kidney transplant recipients. In two separate cohort studies, one adult and one pediatric, serum cystatin C was shown to outperform BTP for the detection of decreased renal function (as measured by inulin clearance); both markers were shown to outperform the serum creatinine level alone.[119,120]

Foster and colleagues have investigated the association of BTP, serum cystatin C, and creatinine-based eGFR with all-cause mortality in a subset of patients from the NHANES cohort.[121] They analyzed data from 6445 adults (enrolled from 1988–1994), with follow-up through December 2006. All three markers were associated with increased mortality

after adjusting for demographics. However, when comparing the mortality risk of the fifth (highest) quintile with the third (middle) quintile, only BTP (HR, 95% confidence interval [CI], 2.14 (1.56–2.94) and serum cystatin C (HR, 1.94 [1.43–2.62]) remained statistically significant (creatinine eGFR, HR 1.31[0.84–2.04]). These effects remained significant when looking at either cardiovascular disease– or coronary heart disease–associated mortality. Similarly, in data from the Atherosclerosis Risk in Communities (ARIC) study. BTP was shown to outperform creatinine-based eGFR (CKD-EPI) in the prediction of mortality and development of kidney failure.[122] More recently, BTP was investigated in a pooled cross-sectional analysis of patients from three separate studies, the Modification of Diet in Renal Disease (MDRD), African American Study of Kidney Disease (AASK), and Chronic Renal Insufficiency Cohort (CRIC).[123] In 3156 patients with CKD, BTP levels were strongly associated with the serum creatinine level and urine protein excretion and demonstrated a correlation with race, where levels were lower in African Americans. Additionally, BTP was clearly associated with advanced age and male gender and inversely related to body mass index.[123]

BTP has also been investigated for its ability to predict and estimate residual kidney function in those undergoing maintenance hemodialysis.[124,125] Using interdialytic urine collections and serum BTP and β_2-microglobulin levels, Wong and colleagues derived equations to estimate GFR and residual urea clearance in 191 patients on hemodialysis.[125] Using the reciprocal of BTP and other factors, they demonstrated an R^2 of 0.70 with GFR and an R^2 of 0.625 with residual urea clearance. Similarly, Shafi and colleagues developed separate equations in 44 dialysis patients completing 24-hour urine tests and went on to validate their equations in a cohort of 826 patients from the Netherlands Cooperative Study on the Adequacy of Dialysis.[124] They demonstrated that BTP provided better accuracy than the traditional markers—creatinine and urea clearance—and that BTP had an AUC of 0.82 for detecting the gold standard–measured urinary urea clearance. Thus, BTP and other markers show promise as a method to estimate residual renal function in dialysis patients.

Concentrations of BTP are not affected by commonly used immunosuppressive medications, such as prednisone, mycophenolate mofetil, and cyclosporine.[126] This is especially useful when evaluating kidney function in kidney transplant recipients, in whom cystatin C concentrations may be falsely elevated due to steroid treatment.[127] Unlike for serum creatinine values, age and race were not associated with BTP concentrations. Several GFR estimation equations based on BTP have been developed for use in kidney transplant recipients.[126,127] However, these equations, similar to those discussed previously for dialysis patients, will require external validation in larger and more diverse patient groups. In contrast to creatinine, one limitation of using BTP is lack of widespread availability and standardization of the assay.

URINARY GLOMERULAR CELL INJURY MARKERS

Defects in podocyte structure have been reported in many glomerular diseases, which have been classified as "podocytopathies."[128,129] Injured podocytes have been reported in immunologic and nonimmunologic forms of human glomerular disease, including hemodynamic injury, protein overload states, injury from environmental toxins, minimal change disease, focal segmental glomerulosclerosis, membranous glomerulopathy, diabetic nephropathy, and lupus nephritis.[130–135] Podocytes may be injured in many forms of human and experimental primary glomerular disease and in secondary forms of focal segmental glomerulosclerosis, including those caused by hypertension, diabetes, and tubulointerstitial disease.[136–138] Before detachment from the glomerular basement membrane, podocytes undergo structural changes, including effacement of foot processes and microvillous transformation.[128,129,139,140]

PODOCYTE COUNT

After undergoing the aforementioned structural changes, podocytes detach from the glomerular basement membrane and are excreted into the urine. Urinary levels of viable podocytes have been extensively studied in several renal diseases.[141–144] Numerous studies have reported that the number of podocytes shed in patients with active glomerular disease is significantly higher than in healthy controls and in patients with inactive disease. Importantly, the podocyte number in urine correlates with disease activity (assessed by renal biopsy) and has been shown to decline with treatment.[145] Studies have linked podocytopenia and disease severity in immunoglobulin (IgA) nephropathy[141,142] and diabetic nephropathy.[143,144] An improved and standardized laboratory method is urgently needed to facilitate measurement of urinary podocyte number. Alternative methods that indirectly assess the number of podocytes in urine include detection of messenger RNA (mRNA) and protein levels of podocyte-specific proteins by polymerase chain reaction (PCR) assay and enzyme-linked immunosorbent assay (ELISA), respectively.

PODOCALYXIN

Podocalyxin is the most commonly used marker protein for detecting podocytes in urine.[146] It is a highly *O*-glycosylated and sialylated type I transmembrane protein of approximately 140 kDa and is expressed in podocytes, hematopoietic progenitor cells, vascular endothelial cells, and a subset of neurons.[146] Podocalyxin participates in a number of cellular functions through its association with the actin cytoskeleton, ezrin, and Na^+/H^+ exchanger regulatory factor 1 and 2 (NHERF1 and NHERF2) proteins. Urinary podocalyxin has been reported as a marker of activity in a number of diseases, including IgA nephropathy, Henoch-Schönlein purpura, diabetic nephropathy, lupus nephritis, poststreptococcal glomerulonephritis, membranous nephropathy, focal segmental glomerulosclerosis, and preeclampsia.[147–155] Unfortunately, because podocalyxin is expressed on a number of cell types, the presence of podocalyxin in the urine is not always reflective of urinary podocytes.

URINARY TUBULAR INJURY MARKERS

Microscopic examination of the urine has been used for many years to gain insight into the degree of glomerular and tubular injury. Other components of the urine have been used to quantitate tubular cell injury in a more specific and sensitive fashion. These markers have been demonstrated to be extremely valuable in detecting kidney injury in the setting of AKI. Moreover, some of these biomarkers, such as

interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), neutrophil gelatinase–associated lipocalin (NGAL), and liver-type fatty acid binding protein (L-FABP), have been shown to be potentially useful in a variety of contexts in acute and chronic kidney injury. In this section, the utility of urine microscopy is described briefly. and some of the emerging biomarkers of tubular injury are discussed.

URINE MICROSCOPY

Urine microscopy with sediment examination is a time-honored test that is routinely used to assist in the diagnosis of kidney injury.[156–158] The urine from patients with tubular injury typically contains proximal tubular epithelial cells, proximal tubule epithelial cells casts, granular casts, and mixed cellular casts. Patients with predominantly prerenal azotemia occasionally have hyaline or fine granular casts in their urine.[159–161] Several studies have shown that the increase in urinary cast excretion correlates well with AKI.[160,162,163] Marcussen and associates[162] have demonstrated that patients with tubular injury have a high number of granular casts compared with those with prerenal azotemia.

There has been a resurgence of urinalysis sediment scoring systems for the diagnosis of AKI.[161,164] Several of these systems have shown excellent specificity for AKI and correlate well with the severity of AKI.[164–166] However, their widespread acceptance has been hampered by the relatively modest sensitivity of urine microscopy for detecting AKI.[161,164,167,168] Urine microscopy remains a user-dependent tool that displays a tremendous amount of interphysician variability, which likely contributes to its suboptimal sensitivity for AKI.[169] Three of the most widely reported urine microscopy scoring systems are reviewed in Table 27.6.

Several other studies have looked at the potential of using urine microscopy in combination with other biomarkers for detecting tubular injury, with varying degrees of success.[165–167] In the near future, urine microscopy, a current mainstay in the clinical diagnosis of AKI, could be used in concert with markers of glomerular function and validated biomarkers of tubular injury to diagnose AKI.

Table 27.6 Review of Urine Microscopy Scoring Systems

Study	Scoring System
Chawla et al. 2008[164]	Grade 1: No casts or RTE Grade 2: At least one cast or RTE but <10% of LPF Grade 3: Many casts or RTEs (from 10%–90% of LPF) Grade 4: Sheet of muddy brown casts and RTEs in >90% of LPF
Perazella et al. 2010[165]	0 points: No casts or RTE seen 1 point each: One to five casts/LPF or one to five RTEs/HPF 2 points each: Six or more casts/LPF or ≥six RTEs/HPF
Bagshaw et al. 2011[167]	0 points: No casts or RTE seen 1 point each: One cast or one RTE/HPF 2 points each: Two to four casts or RTEs/HPF 3 points each: Five casts or more or five or more RTEs/HPF

HPF, High-power field; *LPF,* low-power field; *RTE,* renal tubule epithelial cells.

α_1-MICROGLOBULIN

α_1-Microglobulin is a low-molecular-weight glycoprotein of approximately 27 to 30 kDa and is a member of the lipocalin superfamily. α_1-Microglobulin is primarily synthesized by the liver and is available in free form and as a complex with IgA.[170] α_1-Microglobulin has been detected in human serum, urine, and cerebrospinal fluid. Urine and serum levels have been found to be elevated in patients with renal tubular diseases. α_1-Microglobulin is freely filtered at the glomerulus and completely reabsorbed and catabolized by the normal proximal tubule. Megalin mediates the uptake of this protein in the proximal tubule. Therefore, an increase in the urinary concentration of α_1-microglobulin indicates proximal tubular injury or dysfunction.

The urinary levels of α_1-microglobulin are influenced by age. The normal range in populations younger than 50 years of age is less than 13 mg/g of creatinine and, in those 50 years of age and older, is less than 20 mg/g of creatinine.[170] Unlike β_2-microglobulin, α_1-microglobulin is more stable over a range of pH levels in the urine,[171] which makes it a more acceptable urinary biomarker.

Acute Kidney Injury

α_1-Microglobulin quantitation in the urine has been reported as a sensitive biomarker for proximal tubule dysfunction in adults and children.[170,172] In a small cohort of 73 patients, of whom 26 required renal replacement therapy, Herget-Rosenthal and colleagues compared levels of α_1-microglobulin, β_2-microglobulin, cystatin C, retinol binding protein, α-glutathione S-transferase, lactate dehydrogenase, and *N*-acetyl-β-D-glucosaminidase (NAG) early in the course of AKI.[173] They found that urinary cystatin C and α_1-microglobulin had the highest ability to predict the need for renal replacement therapy. In this study, urinary α_1-microglobulin had an AUC of 0.86 for predicting the need for renal replacement therapy. This is similar to the results of a Zheng and colleagues,[174] who measured α_1-microglobulin levels in 58 children undergoing cardiac surgery and found that levels were higher in those who developed AKI (AKIN criteria). Four hours after cardiopulmonary bypass, α_1-microglobulin had an AUC of 0.84 (0.72–0.95), with a value of 290 mg/g providing a sensitivity of 90% and specificity of 79%.However, follow-up studies have reported mixed results, with Martensson and colleagues[175] finding no difference in α_1-microglobulin levels between those with and without AKI in the setting of sepsis and septic shock in a small prospective single-center study of 45 subjects.

More recently, α_1-microglobulin levels at the time of arrival to the emergency room (ER) have demonstrated the ability to correlate with the development of AKI, with an AUC of 0.88 and a cutoff value of 35 mg/g providing reasonable sensitivity (80%) and specificity (81%). However, α_1-microglobulin did not remain an independent predictor of AKI in the multivariable model (OR [95% CI], 1.85 [0.80–4.31]).[176] In addition, α_1-microglobulin has also been reported as a useful marker for proximal tubular damage, and recovery in early infancy and has been shown to correlate with tubular atrophy and interstitial fibrosis on renal transplant biopsy 1 year after transplantation.[177,178]

Chronic Kidney Disease

Increasingly, α_1-microglobulinin has been investigated in the setting of CKD. Limited studies have demonstrated that this marker may correlate with disease activity and proximal tubule damage in the setting of diabetic nephropathy, as well as idiopathic membranous nephropathy.[179,180] In a cohort of 2948 Framingham Heart Study participants, O'Seaghdha and colleagues[181] demonstrated that although α_1-microglobulin does not correlate with the development of CKD or albuminuria during the mean of 10.1 years follow-up, it does correlate with all-cause mortality (HR, 1.26 [1.13–1.40; $P < .001$]. In a different cohort, Jotwani and colleagues have demonstrated that α_1-microglobulin levels in women infected with human immunodeficiency virus (HIV) are associated with both kidney function decline and mortality.[182] Compared with those with the lowest α_1-microglobulin levels, those with the highest levels had a 2.1 (1.3–3.4)-fold increased risk of developing CKD and a 2.7-fold risk of a 10% decline of their eGFR. This correlation in CKD development and progression was separate from the 1.6-fold adjusted risk of mortality, which accounted for baseline kidney function as well as the presence of albuminuria.[182] Follow-up studies in this same cohort did not link α_1-microglobulin levels with an *APOL1* genotype.[183]

Despite these recent data on urine concentration, the use of α_1-microglobulin remains limited due to prior studies demonstrating variation in serum levels with age, gender,[184] and clinical conditions, including liver disease,[170] ulcerative colitis,[185] HIV infection, and mood disorders,[170] as well as the lack of international standardization. Urinary α_1-microglobulin is measured by an immunonephelometric assay.

β_2-MICROGLOBULIN

β_2-Microglobulin is a low-molecular-weight polypeptide with a molecular weight of 11.8 kDa. β_2-Microglobulin is present on the cell surface of all nucleated cells and in most biologic fluids, including serum, urine, and synovial fluid. β_2-Microglobulin is normally excreted by glomerular filtration, reabsorbed almost completely (≈99%), and catabolized by the normal proximal tubule in humans.[186,187] Megalin mediates the uptake of this protein in the proximal tubule.[187] In healthy individuals, approximately 150 to 200 mg of β_2-microglobulin is synthesized daily, with a normal serum concentration of 1.5 to 3 mg/L. Any pathologic state that affects kidney tubule function will result in an increase in β_2-microglobulin levels in the urine because of the impeded uptake of β_2-microglobulin by renal tubular cells. For spot urine collections, the concentration of β_2-microglobulin in healthy individuals is typically 160 μg/L or less or 300 μg/g of creatinine or less. Unlike urea, its serum levels are not influenced by food intake, which makes it an attractive marker for malnourished patients with low serum urea levels. In patients with CKD, increases in serum β_2-microglobulin levels reflect a decrease in glomerular function. In the aforementioned cross-sectional study of patients from the MDRD, AASK, and CRIC studies, serum β_2-microglobulin levels were weakly associated when male gender and non–African American race were compared; however, unlike other markers, it was strongly associated with smoking. Thus, serum β_2-microglobulin is clearly affected by nonrenal variables, demonstrating some of the limitations when implementing β_2-microglobulin as a marker of GFR.[123]

In ESRD patients, serum levels of β_2-microglobulin are usually in the range of 20 to 50 mg/L. β_2-Microglobulin accumulation is linked to toxicity because the molecule precipitates and forms fibrillary structures and amyloid deposits, particularly in bone and periarticular tissue, which leads to the development of carpal tunnel syndrome and erosive arthritis.[187,188] Elevated serum levels of β_2-microglobulin have been reported in several AKI and CKD clinical settings, including cadmium toxicity,[189] following cardiac surgery,[190,191] liver transplantation,[192] and renal transplantation.[193] In idiopathic membranous nephropathy, the β_2-microglobulin level was identified as a superior independent predictor of the development of GFR decline.[194] Other studies have reported that β_2-microglobulin performs as well as, if not better than, the serum creatinine level for the detection of AKI in critically ill children[195] or following adult cardiac surgery.[191]

Serum concentrations of β_2-microglobulin should be interpreted cautiously because they are altered significantly in various diseases, including rheumatoid disorders and several types of cancers.[196,197] Initially, it was believed that the increase in β_2-microglobulin levels in CKD is solely due to declines in kidney function, but some studies have shown that other factors, including increased synthesis of β_2-microglobulin, may contribute in patients with ESRD.[198] Another significant drawback associated with the use of urinary β_2-microglobulin as a marker of kidney injury is its instability in the urine at room temperature, particularly when the pH is less than 5.5. Because of this, the urine should be alkalinized and frozen at −80°C immediately after collection.[188,199]

HEPCIDIN-25

Hepcidin-25 is a 2.8-kDa hormonal regulator of iron metabolism produced in the liver, heart, and kidney. Hepcidin-25 binds to and induces the internalization and degradation of the transmembrane iron exporter ferroportin.[200] Hepcidin-25 acts to downregulate iron uptake and reduce extracellular iron availability from stored iron.[201] Given its link to iron metabolism, and the fact that free iron is known to be released in the setting of the ischemia reperfusion injury and oxidative stress that occur with cardiopulmonary bypass, urinary hepcidin-25 has been investigated as a marker of kidney injury following cardiac surgery. Ho and colleagues[202] have identified urinary hepcidin-25 in a nested case-control study of 44 adults who underwent cardiac surgery. Using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS), urine from 22 individuals who developed at least RIFLE risk AKI and 22 individuals whose creatinine level did not increase more than 10% from baseline during the postoperative period (no AKI), they demonstrated that hepcidin-25 is dramatically upregulated in the urine of those with no AKI. Taking this a step further, this same group quantified the concentration of hepcidin-25 in the urine (normalized to urine creatinine) and demonstrated that concentrations were higher in those who did not go on to develop postoperative AKI ($P < .0005$).

In a multivariable analysis, hepcidin-25 was significantly associated with the avoidance of AKI, with urinary concentrations on postoperative day 1 showing an AUC of 0.80.[203] The data from this small study have been corroborated by another modestly sized cohort of 100 adults undergoing cardiopulmonary bypass (CPB). Haase-Fielitz and colleagues have demonstrated that 6 hours after CPB, urinary hepcidin-25 levels are lower in the 9 subjects who developed RIFLE-based

AKI compared with those with no AKI (AUC = 0.80; P = .004).[204] More recently, Prowle and colleagues measured hepcidin-25 in a 93-subject multicenter cohort of patients undergoing cardiac surgery with CPB.[205] Although this study did not have a 6-hour CPB time point, it did demonstrate that hepcidin -25 values 24 hours after surgery were different in those with and without AKI ($P < .001$), with values being higher in those without AKI. Additionally, they demonstrated that combining hepicidin-25 with postoperative NGAL values leads to an AUC of 0.84 for the development of RIFLE- based AKI. In addition to establishing a universal commercially available assay, these results warrant further preliminary investigations in other AKI settings, as well as prompt validation in a larger cohort of cardiac surgery patients.

INTERLEUKIN-18

IL-18 is an 18-kDa proinflammatory cytokine that is activated by caspase-1 and produced by renal tubule cells and macrophages. Animal studies have indicated that IL-18 is a mediator of acute tubular injury, including both neutrophil and monocyte infiltration of the renal parenchyma.[206,207] In studies using caspase-1 knockout mice, these mice experienced the same degree of ischemic AKI as wild-type mice injected with an IL-18 neutralizing antiserum, demonstrating that IL-18 is an important mediator of ischemic AKI.[207]

Other studies have shown that IL-18 plays a major role in macrophage activation, with mice engrafted with IL-18–deficient bone marrow experiencing a lower incidence of AKI than those with IL-18–replete marrow.[208] Similarly, in IL-18 knockout mice with AKI, tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase, macrophage inflammatory protein-2, and monocyte chemoattractant protein-1 messenger RNA expression are all decreased, indicating the deleterious impact of IL-18 on AKI. In the human kidney, IL-18 is induced and cleaved mainly in the proximal tubules and released into the urine. IL-18 has been shown to participate in a variety of renal disease processes, including ischemia reperfusion injury, allograft rejection, infection, autoimmune conditions, and malignancy. IL-18 is easily and reliably measured in the urine by commercially available ELISA and microbead-based assays.

Acute Kidney Injury

Several studies have demonstrated the usefulness of IL-18 as a biomarker for the detection of AKI. Originally, Parikh and associates studied a group of 72 patients and reported urinary IL-18 levels to be significantly higher in patients diagnosed with acute tubular necrosis than in patients with prerenal azotemia or urinary infection or in healthy control subjects with normal renal function.[209] Since then, several large multicenter studies have gone on to investigate the ability of IL-18 to detect AKI in a variety of clinical settings.

The Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) consortium measured IL-18 in 1219 adults who underwent cardiac surgery. The study selected those at high risk for postoperative AKI because of the presence of one of the following: emergency surgery, preoperative serum creatinine level >2 mg/dL, left ventricular ejection fraction <35%, stage III or IV New York Heart Association cardiac failure, age >70 years, preexisting diabetes mellitus, concomitant coronary artery bypass grafting (CABG), and valve surgery or repeat cardiac surgery. Those with preoperative AKI, kidney transplants, ESRD, or a preoperative serum creatinine level >4.5 mg/dL were excluded. After dividing the cohort into quintiles, the highest quintile of IL-18 was associated with a 6.8-fold higher risk of AKI, defined as a postoperative doubling of the serum creatinine level or undergoing acute dialysis.[210] The first postoperative concentration of IL-18 (0–6 hours) had an AUC of 0.74 that increased to 0.76 after combining IL-18 with a clinical model of factors known to affect AKI risk. The TRIBE-AKI pediatric cohort (311 children) reported results in line with the TRIBE adult study, in which the highest quintile was associated with 9.4-fold increased risk of developing AKI (doubling or dialysis) compared with the first quintile. The affect was slightly attenuated after adjusting for clinical factors known to affect AKI (adjusted odds ratio [OR], 95% CI, 6.9 [1.7–28.8]).[211]

Urine IL-18 displayed a similar trend when investigating the association between biomarker concentrations and duration of AKI.[212] Stratifying the cohort of those with AKI according to the duration, 61.7% (n = 251) had a duration of 1 to 2 days, 28.9% (n = 118) had a duration of 3 to 6 days, and 9.3% (n = 38) had a duration >7 days. The fifth quintile of IL-18 was strongly associated with the duration of AKI, providing an unadjusted OR of 3.90 (2.62–5.78). Although this effect was attenuated after adjusting for clinical factors known to affect AKI, it remained significant, at 2.90 (1.80–4.68).[212]

In another secondary analysis of the TRIBE-AKI adult cohort, Koyner and colleagues[213] have demonstrated that IL-18 at the time of AKI can forecast those with early AKI (AKIN stage 1) who go on to progress to more severe stages of AKI (AKIN stage 2 or 3). In the 380 adults who developed at least stage 1 AKI, 45 went on to progress to stage 2 or 3. In the entire cohort, the fifth quintile of IL-18 was at increased risk for developing progressive AKI (3.63 [1.64–.03]), and this effect was only slightly attenuated after adjusting for the clinical model (3.00 [1.25–7.25]).

When investigated in a separate secondary analysis of this cohort, IL-18 concentrations collected in the immediate postoperative period were associated with long-term mortality following cardiac surgery. This investigation provided a median follow-up of 3.0 years (interquartile range [IQR], 2.2–3.6) during which 139 of the 1199 subjects died (50 deaths/1000 person-years). After adjusting for clinical factors known to affect mortality, patients without AKI (n = 792) in the third tertile of IL-18 were at increased risk of long-term mortality compared with the first tertile (adjusted HR, 1.23 [1.02–1.48]). This effect was magnified in subjects with perioperative AKI (n = 407), with the third tertile having an adjusted HR of 3.16 (1.53–6.53) compared with the reference cohort. Thus, IL-18 provides additional prognostic information about long-term postoperative mortality in patients with and without AKI.[214]

In the setting of critical illness and ICU admission, IL-18 has not demonstrated the same robust results. In a study of 451 critically ill subjects, 86 of whom developed AKI within the first 48 hours, Siew and colleagues have demonstrated that urine IL-18 does not reliably predict AKI.[215] Although IL-18 levels at the time of ICU admission were higher in those who went on to develop AKI, the AUC was 0.62 (0.54–0.69) and only marginally improved to 0.67 after the exclusion of those with prior known CKD (eGFR < 75 mL/min). Despite the inability to detect AKI reliably, urine IL-18

levels did correlate with other adverse patient outcomes, including the need for renal replacement therapy (RRT) and 28-day mortality. This poor performance in the setting of critical illness has been corroborated by others, including data from a post hoc analysis of the EARLY ARF trial. In this prospective observational study in two large general ICUs ($n = 529$), IL-18 had an AUC of only 0.62 for the diagnosis of AKIN stage 1 AKI, but once again performed much better at forecasting the need for RRT or death (within 7 days). Unlike other biomarker studies, this IL-18 study did not demonstrate improved predictive powers, with the cohort stratified according to preadmission CKD stage.[216] In a separate post hoc analysis of this same EARLY ARF cohort, urinary IL-18 concentrations were shown to be significantly higher in those with prerenal azotemia ($n = 61$, defined as AKI that recovered within 48 hours of ICU admission, and was associated with a fractional excretion of sodium <1%), compared with those with no AKI ($n = 285$). There was a trend toward higher values in those with AKI ($n = 114$, nonprerenal) compared with those with prerenal AKI ($P = .053$).[217]

In a single-center study of 339 mixed surgical and medical ICU patients, Doi and colleagues have demonstrated that IL-18 levels are significantly elevated in those with both established and newly diagnosed AKI at the time of ICU arrival.[218] Although biomarker concentrations and AUCs were higher in those with established AKI (AUC, 0.78) compared with newly diagnosed AKI (AUC, 0.59), both these subgroups were significantly different compared with the no-AKI cohort. In this same study, IL-18 levels were significantly higher in nonsurvivors. These results were fairly similar to those of Nickolas and colleagues,[219] who measured urinary biomarkers in 1635 ER patients at the time of admission and compared the values to adjudicated AKI outcomes, in which prerenal AKI was defined as RIFLE Risk that returned to baseline within 72 hours and in the clinical setting, suggested decreased transient effective circulating volume. They demonstrated that IL-18 values for those with more severe intrinsic AKI were significantly higher compared with those with prerenal AKI. However, there was no difference between those with prerenal AKI and no AKI.

At kidney transplantation, the IL-18 level accurately identified delayed graft function (DGF) (AUC = 0.90) and predicted the rate of increase in serum creatinine concentration.[220] To understand the utility of IL-18 and urinary NGAL in predicting graft recovery after kidney transplantation, Hall and colleagues[222] conducted a prospective, multicenter, observational cohort study of recipients of deceased donor kidney transplants. They collected serial urine samples from 91 patients for 3 days after transplantation. After adjustment for recipient and donor age, cold ischemia time, urine output, and serum creatinine concentration, NGAL and IL-18 concentrations accurately predicted the need for dialysis in transplant recipients. Furthermore, NGAL and IL-18 concentrations also predicted graft recovery up to 3 months later.[222] In further follow-up of this cohort, urine IL-18 concentrations collected at the time of surgery were correlated with 1-year posttransplantation graft outcomes. Above-median values of IL-18 on the first postoperative day had an adjusted OR of 5.5 (1.4–21.5, 95% CI) with poor graft function, defined as a GFR < 30 mL/min or return to RRT.[223]

In a study by Ling and associates involving patients who underwent coronary angiography, urinary IL-18 and NGAL concentrations were significantly increased at 24 hours postprocedure in those who developed contrast-induced nephropathy, but not in the control group. [224] ROC curve analysis demonstrated that both IL-18 and NGAL showed better performance in the early diagnosis of contrast nephropathy than the serum creatinine level ($P < .05$). Importantly, elevated urinary IL-18 concentrations 24 hours after contrast administration were also found to be an independent predictive marker for later major cardiac events (relative risk, 95% CI, 2.09 [1.15–3.77]).

Chronic Kidney Disease

There are promising data about IL-18 in the setting of CKD. In patients with diabetic kidney disease and proteinuria, IL-18 levels in renal tubular cells are higher than in patients with nondiabetic proteinuric disease.[221] In the Women's Interagency HIV study, urine IL-18 levels were independently associated with a more rapid loss of renal function after multivariable adjustment.[225] In this cohort study of 908 HIV-infected women, urine IL-18 was the only biomarker (KIM-1 and albumin-to-creatinine ratio [ACR]) were also measured) that was associated with worsening renal function over time, as measured by eGFR–cystatin C. Urine IL-18 predicted an increased relative risk of renal function decline between 1.4 and 2.16, depending on the models used. In a follow-up study, the same group measured urine IL-18 in 908 HIV- infected and 289 noninfected women in the Women's Interagency HIV Study.[226] In this cross-sectional cohort study, they demonstrated that after multivariable adjusted linear regression analysis that IL-18 concentrations are significantly higher in those with HIV (38%; $P < .0001$). They subsequently demonstrated this same phenomenon in a cross-sectional study of 813 HIV-infected men and 331 uninfected men, with urine IL-18 being 52% higher in those with HIV.[227] Returning the female cohort, they demonstrated that urine IL-18 concentrations are significantly associated with higher HIV RNA levels and lower CD4 counts, hepatitis C infection, and high-density lipoprotein cholesterol levels, thus indicating a more extensive role for IL-18 in the setting of HIV-related kidney care.[226] This promising HIV data are in contrast to those of the Consortium for Radiologic Imaging for the Study of Polycystic Kidney Disease (CRISP), which measured IL-18 in 107 patients with autosomal dominant polycystic kidney disease and found that although there was an increased mean IL-18 over the 3-year follow-up period, there was no association between tertiles of IL-18 and change in total kidney volume or eGFR.[228]

KIDNEY INJURY MOLECULE-1

Kidney injury molecule-1 (KIM-1 in humans, Kim-1 in rodents), which is also referred as "T-cell immunoglobulin and mucin domain–containing protein-1" (TIM-1) and "hepatitis A virus cellular receptor-1" (HAVCR-1), is a type I transmembrane glycoprotein with an ectodomain containing a six-cysteine immunoglobulin-like domains, two *N*-glycosylation sites, and a mucin domain. In an effort to identify molecules involved in kidney injury, Ichimura and colleagues originally discovered Kim-1 using representational difference analysis (a PCR-based technique) in rat models of acute ischemic kidney injury.[229,230] Importantly, KIM-1 was shown to be significantly expressed in kidneys, specifically in proximal tubular cells of humans after ischemic injury, whereas it was virtually

absent or present at low levels in healthy kidneys. KIM-1 has evolved as a marker of proximal tubular injury, the hallmark of virtually all proteinuric, toxic, and ischemic renal diseases. KIM-1 has been shown to be a highly sensitive and specific marker of kidney injury in several rodent models, including models of injury due to ischemia,[229,231] cisplatin, folic acid, gentamicin, mercury, chromium,[232,233] cadmium,[234] contrast agents,[235] cyclosporine,[236] ochratoxin A, aristolochic acid, D-serine, and protein overload.[237]

In 2002, Han and associates[231] published the first clinical study linking urinary levels of KIM-1 with AKI, demonstrating that tissue expression of KIM-1 is correlated with the severity of acute tubular necrosis and corresponding levels of KIM-1 ectodomain in the urine of patients with clinically significant AKI. Since then, numerous other studies have been published on the ability of KIM-1 to detect AKI in a variety of settings, including cardiac surgery, critical illness, and general hospitalized AKI, with mixed success.[44,216,217,238–244]

KIM-1 has been investigated in several multicenter larger trials. In the TRIBE-AKI adult and pediatric cardiac surgery cohorts, the fifth quintile of urinary KIM-1 values was associated with an increased risk of AKI (those with postoperative doubling were at a 6.2-fold increased risk of AKI compared with those with the lowest KIM-1 values). This risk remained significant (4.8-fold) after adjusting for the clinical model—age, race, gender, CPB time, nonelective surgery, preoperative GFR, diabetes, hypertension, and study center. The effect was completely attenuated after including urinary IL-18 and plasma and urine NGAL into the model.[243] Urine KIM-1 concentrations were associated with duration of AKI (categories: 1 to 2 days, 3 to 6 days, and >7 days). Compared with the first quintile, the fifth quintile of early postoperative KIM-1 provided an unadjusted OR of 2.96 (2.01–4.37) for longer duration of AKI. After adjusting for clinical factors known to affect AKI (including but not limited to age, gender, race, CPB time, baseline kidney function, diabetes, and hypertension), the OR remained statistically significant 2.30 (1.51–3.53).[212]

When looking at long-term mortality in the TRIBE cohort, the third tertile of perioperative urine KIM-1 concentrations was associated with increased mortality. In those without AKI (n = 792), the third tertile had an adjusted HR of 1.83 (1.44–2.33) for mortality, whereas in the 407 subjects with AKI, the adjusted HR was slightly higher 2.01(1.31–3.1).[214] In the pediatric cohort, those in the fifth quintile of urine KIM-1 were at increased risk of AKI in the unadjusted analysis; however, this effect was attenuated and no longer significant after multivariable adjustment.[243]

The data on urine KIM-1 in the setting of critical illness–related AKI have been just as mixed as the perioperative results, with KIM-1 having an AUC of 0.66 (95% CI; 0.61–0.72) for the diagnosis of AKI in samples from the EARLY ARF study. The results were less impressive with regard to the prediction of dialysis (0.62) or death (0.56) within the first week following ICU admission.[216] On the other hand, in a cohort of 529 mixed ICU patients, urine KIM-1 outperformed other biomarkers in its ability to detect AKI at the time of ICU admission in those with a preadmission GFR < 60 mL/min (AUC = 0.70, [0.58 –0.82]). In a separate post hoc analysis of this cohort, KIM-1 levels demonstrated a significant stepwise increase when comparing those with no AKI (median, 170 μg/mmol creatinine [Cr]; interquartile range [IQR], 69 –445) to those with prerenal AKI (291 μg/mmol Cr; IQR, 121–549) and those with intrinsic AKI (lasting more than 48 hours; 376 μg/mmol Cr; IQR, 169–943).[217] In addition, urine KIM-1 was able to forecast the development of intrinsic AKI at the time of ER arrival with an AUC of 0.71 (0.65–0.76; $P < .001$). KIM-1 values again increased in a stepwise fashion, with values being lowest in those with no AKI or CKD < stable CKD < prerenal AKI < intrinsic AKI. Also, KIM-1 values were able to forecast the need for RRT, as well as inpatient mortality.[219]

The usefulness of KIM-1 has been demonstrated not only as a urinary marker but also as a tool for evaluating kidney injury in kidney biopsy specimens by immunohistochemical methods. For example, Van Timmeren and associates found that the level of KIM-1 protein expression in proximal tubule cells correlated with tubulointerstitial fibrosis and inflammation in kidney tissue specimens from 102 patients who underwent kidney biopsy for a variety of kidney diseases.[245] In a subset of patients whose urine was collected near the time of biopsy, urinary KIM-1 levels were significantly correlated with tissue KIM-1 expression in biopsy samples from patients with deterioration in kidney function and histologic changes indicative of tubular damage. In biopsy specimens from transplanted kidneys, increased KIM-1 staining was seen in 100% of patients with a deterioration of kidney function and pathologic changes indicating tubular injury, in 92% of patients with acute cellular rejection, and in 28% of patients with normal biopsy findings.[246] This is in contrast to the findings of Hall and associates, who have demonstrated that urinary KIM-1 levels do not correlate with early posttransplantation or 1-year graft function.[223,241] Similarly, Schroppel and colleagues have investigated KIM-1 RNA expression in pretransplantation biopsies collected from both living and deceased donor kidneys and found no significant correlation between KIM-1 staining and the occurrence of DGF.[247]

Chronic Kidney Disease

KIM-1 also shows promise as a useful biomarker in CKD. In addition to serving as a marker of proximal tubule dysfunction, animal studies have demonstrated that KIM-1 is upregulated in the later phases of AKI and plays an important role in renal repair; thus, it may be a major player in the pathophysiology of repair and CKD development after AKI.[248] The ability to serve as a marker of CKD was evident in a nested case-control study from 686 participants from the Multi-Ethnic Study of Atherosclerosis (MESA). Cases were defined as those with a baseline eGFR > 60 mL/min who subsequently developed CKD stage 3 and/or had a rapid drop in kidney function over the 5-year study period. Each doubling of KIM-1 level (in pg/mL) was associated with a 1.15 (1.02–1.29) increased odds of developing CKD stage 3 or a rapid decline in GFR.

Similarly, at study entry, those in the highest decile of KIM-1 had a twofold increased risk of this same endpoint compared with the lower 90%. This ability to predict the development and progression of CKD was independent of the presence of albuminuria.[249] Similarly, when investigated in a cohort of 149 persons with chronic congestive heart failure during 5 years of follow-up, KIM-1 levels were strongly associated with the progression of CKD (defined as a >25% drop in eGFR from baseline; $P < .05$).[250] Urine KIM-1 and NAG were both independent predictors of the combined endpoint of CKD progression and all-cause mortality.[250] In a multivariable Cox proportional hazard model that included

age, gender, body mass index (BMI), history of diabetes, hypertension, stroke, baseline eGFR, albuminuria, left ventricular ejection fraction, diuretic use, and brain natriuretic peptide levels, baseline eGFR, diuretic use, and KIM-1 and NAG levels were the only independent predictors of both these outcomes. More recently, in a pooled multivariable-adjusted analysis of five unique cohort studies, log-transformed urine KIM-1 levels (normalized to urine creatinine) were found to be higher in current smokers, and lower in blacks and those patients receiving angiotensin-converting enzyme inhibitors (ACE-Is) or angiotensin receptor blockers (ARBs).[251] Similarly, KIM-1 levels were higher in those with lower eGFRs and higher in those with albuminuria.

These results, which indicate the utility of KIM-1 as a biomarker for CKD, are in contrast to those of Bhavsar and colleagues, who measured KIM-1 in a case-control substudy of the ARIC study.[252] In this study of 286 subjects, 143 of whom developed new-onset CKD stage 3, KIM-1 did not display the ability to forecast or diagnose those at risk for CKD development or progression. In a follow-up case-control study by Foster and colleagues of 135 patients with ESRD and 186 controls from the same ARIC cohort (study visit 4), it was found that urinary KIM-1, normalized to urine creatinine, was associated with an increased risk of ESRD (unadjusted OR, 2.24 [1.97–4.69]; $P = .03$).[253] However, this effect was attenuated when adjusting for other factors that increase the risk for ESRD (e.g., age, baseline albuminuria, baseline eGFR).

A recent study of the 2466 participants in the Chronic Renal Insufficiency Cohort Study (CRIC) reported findings similar to those of Foster and colleagues[253] on the role of KIM-1 as a biomarker of CKD progression.[254] In this study, with 9433 person-year follow-up, there were 581 cases of CKD progression defined as incident ESRD or a 50% decrease in eGFR. All four upper quintiles of KIM-1 were associated with increased risk of progression (compared with the lowest quintile) in unadjusted analyses (e.g., fifth quintile HR of 7.68 [5.61–10.5]). However, this effect was completely attenuated after the addition of other variables known to associate with CKD progression (e.g., age, gender, race, albuminuria, diabetes, cardiovascular disease).[254]

KIM-1 has been investigated and shown promise in a variety of other clinical settings, including in children with chronic renal tubular damage from vesicoureteral reflux,[25] HIV nephropathy,[225,256] posttransplantation DGF,[257] and diabetic nephropathy.[258,259] In patients with IgA nephropathy, urinary KIM-1 levels were significantly higher than in healthy controls. Furthermore, the levels of urinary KIM-1 correlated positively with serum creatinine concentration and proteinuria and correlated inversely with creatinine clearance. Similarly, tubular KIM-1 expression, as determined by immunohistochemical analysis, correlated closely with urinary levels ($r = 0.553$; $P = .032$).[260] Sundaram and associates have evaluated the potential of KIM-1, L-FABP, NAG, NGAL, and transforming growth factor-β1 (TGF-β1), together with conventional renal biomarkers (urine albumin level, serum creatinine concentration, and serum cystatin C–GFR), to detect nephropathy early in patients with sickle cell anemia. Only KIM-1 and NAG showed a strong correlation with albuminuria; other markers did not show any association with albuminuria.[261]

More recently, plasma KIM-1 was measured in two distinct cohorts at risk for diabetic kidney disease.[262] Plasma KIM-1 was measured in subjects from the Action to Control Cardiovascular Risk in Diabetes (ACCORD) and Veterans Administration NEPHROpathy iN Diabetes (VA-NEPHRON-D) cohorts. In the ACCORD cohort, baseline KIM-1 levels were higher in those who went on to develop incident CKD. Similarly, in the NEPHRON-D study, those who went on to develop progressive CKD had higher levels at the start of the study (median IQR, 735 pg/mL [438–1172]) compared with 373 pg/mL (225–628; $P < .001$).[262] Those in the highest quartile of KIM-1 concentrations in both studies were more likely to experience adverse renal outcomes compared with those in the lowest quartiles of KIM-1 concentrations. This work expands on the limited data that link plasma KIM-1 with CKD progression.[262] Although the exact mechanism whereby KIM-1 enters the plasma circulation in the setting of renal injury remains unclear, these promising studies will undoubtedly lead to further investigation of the link between plasma KIM-1 and CKD.

LIVER-TYPE FATTY ACID BINDING PROTEIN

Urinary fatty acid binding protein 1 (FABP1) has been proposed to be a useful biomarker for early detection of AKI and monitoring of CKD. Also known as "L-type" or "liver-type fatty acid binding protein" (L-FABP), FABP1 was first isolated in the liver as a binding protein for oleic acid and bilirubin. FABP1 binds selectively to free fatty acids and transports them to mitochondria or peroxisomes, where free fatty acids are beta-oxidized and participate in intracellular fatty acid homeostasis. There are several different types of FABP, which are ubiquitously expressed in a variety of tissues. At this time, nine different FABPs have been reported—liver (L), intestinal (I), muscle and heart (H), epidermal (E), ileal (I1), myelin (M), adipocyte (A), brain (B), and testis (T). L-FABP is expressed in proximal tubules of the human kidney and localized in the cytoplasm. Increased cytosolic L-FABP in proximal tubular epithelial cells may derive not only from endogenous expression, but also from circulating L-FABP that might be filtered at the glomeruli and reabsorbed by tubular cells.

Susantitaphong and associates[263] published a metaanalysis reporting the performance of L-FABP from 15 prospective cohorts and two case-control studies. Although the authors were only able to meta-analyze seven of the cohort studies, they demonstrated that L-FABP levels were 74.5% sensitive (60.4–84.8) and 77.6% specific (61.5–88.2) for the diagnosis of AKI. Additionally, they demonstrated that the results were more promising for the prediction of in-hospital mortality. They concluded that based on the low quality of many of the studies and the varied clinical settings, L-FABP may be a promising biomarker for the early detection of AKI.[263] In what follows we highlight some of the larger and more recent clinical investigations of L-FABP.

Portilla and colleagues have demonstrated that L-FABP predicts the development of AKI within 4 hours of surgery in children undergoing cardiac surgery.[264] Others have attempted to validate this finding in the setting of cardiac surgery, with mixed success.[242,265,266] Recently, TRIBE-AKI published the results of the largest study investigating L-FABP in the setting of adult cardiac surgery, which demonstrated that after adjusting for a clinical model that consisted of factors known to affect the development of AKI, L-FABP did not correlate with the development of AKI in either their

pediatric (n = 311) or adult (n = 1219) cohort.[212] It was demonstrated that although L-FABP levels were statistically higher in adults with AKI compared with those with no AKI, the L-FABP concentration (in ng/mL) at the 0- to 6-hour postoperative time point had an AUC of 0.61 for subsequent AKI, and the performance was only marginally better at the 6- to 12-hour time point. Despite this suboptimal performance in detecting incident postoperative AKI, L-FABP was associated with duration of AKI, even after adjusting for factors known to affect the development of AKI. Both the fourth and fifth quintiles were associated with a longer duration of AKI, with adjusted ORs of 1.77 (1.17–2.67) and 1.92 (1.26–2.93), respectively. In the pediatric cohort, although the fifth quintile of L-FABP concentrations at the earliest postoperative time point (0–6 hours) was significantly associated with the development of an AKI odds ratio of 2.9 (1.2–7.1), this effect disappeared after adjustment to 1.8 (0.7–4.6).[243]

On a related note, heart fatty acid binding protein (H-FABP) was also measured in the TRIBE-AKI cohort.[267] H-FABP is a protein that is predominantly expressed in the cytosol of the myocardium, but can be found in the distal tubule and detects cardiac injury in less than 1 hour.[268–270] Log-transformed H-FABP concentrations in the preoperative and immediate postoperative periods were associated with an increased risk of AKI (defined by the AKIN criteria), as well as mortality. In analyses adjusted for factors known to be associated with AKI, preoperative H-FABP values were associated with all stages of AKI (adjusted OR, 2.07 [95% CI, 1.48–2.89]) and mortality 1.67 (1.17–2.37), whereas perioperative values were associated with stage 2 or higher AKI (5.39 [2.87–10.11]) in a similarly adjusted analysis.[267] In view of these promising results, we anticipate further investigation of H-FABP.

Siew and colleagues[271] have reported the performance of L-FABP in 380 critically ill subjects from medical, surgical, trauma, and cardiac ICUs, 130 of whom had AKI defined as AKIN stage 1. L-FABP levels were higher in those with AKI (P = .003) and were able to discriminate incident AKI with an AUC of 0.59 (0.52–0.65). Although L-FABP was not able to predict the composite endpoint of death or RRT, L-FABP was able to predict the need for acute RRT in a multivariable analysis (HR, 2.36 [95% CI, 1.30–4.25]). These findings mirror those of Doi and colleagues, who published a prospective single-center observational cohort study examining the performance of L-FABP in 339 mixed ICU patients.[218] In their study, L-FABP outperformed NGAL, IL-18, NAG, and other biomarkers in the detection of AKI, defined by RIFLE risk. Furthermore L-FABP predicted 14-day mortality with an AUC of 0.90. This study, which followed up a smaller study (n = 145) by this same group of investigators, has paved the way for L-FABP to be validated for clinical use in Japan.[272]

In a cross-sectional study of general hospitalized patients that included 92 participants with AKI and 68 control subjects (26 healthy volunteers and 42 other hospitalized patients, 29 about to undergo coronary catheterization and 13 patients in the ICU with no AKI), Ferguson and colleagues have demonstrated that urinary levels of L-FABP are significantly higher in those with AKI than in hospitalized control patients without AKI, with an AUC of 0.93 (0.88–0.97), with a sensitivity of 83% and specificity of 90% at a cutoff value of 47.1 ng/mg of creatinine.[273] Nickolas and colleagues[219] examined L-FABP at the time of ER arrival and found that it only had fair discriminatory power with regard to AKI. In their cohort of 1635 subjects, L-FABP had an AUC of 0.70 (0.65–0.76); however, there was a clear and significant stepwise increase in L-FABP concentrations across the spectrum of AKI (normal < CKD < prerenal < intrinsic AKI).

Because L-FABP is also expressed by the liver, liver injury can be a potential contributor to increased urinary levels of L-FABP during AKI. However, previous studies in patients with CKD, AKI, and sepsis have shown that serum L-FABP levels do not have an influence on urinary levels and that urinary L-FABP levels are not significantly higher in patients with liver disease than in healthy subjects.[264,274,275]

Urinary L-FABP levels have been investigated as an early diagnostic and predictive marker for contrast medium–induced nephropathy.[276,277] In a study of adult patients with normal serum creatinine concentrations who underwent percutaneous coronary intervention, serum NGAL levels rose at 2 and 4 hours, whereas urinary NGAL and urinary L-FABP levels increased significantly after 4 hours and remained elevated up to 48 hours after cardiac catheterization.[278] Nakamura and associates have demonstrated that baseline urinary L-FABP levels are significantly higher in patients who developed contrast medium–induced nephropathy after coronary angiography; however, the authors did not evaluate the diagnostic performance of urinary L-FABP in predicting AKI.[277]

Finally, L-FABP has been investigated in the setting of renal transplantation, DGF, and postoperative transplant function.[257,279,280] When measured in the deceased donor urine, prior to organ harvesting, or the early postoperative setting, urinary L-FAPB was associated with donor AKI and mildly predictive of DGF. However, although L-FABP concentrations were associated with DGF, they did not outperform postoperative urine output for this endpoint,[280] and they did not predict long-term graft function over the first posttransplantation year.[257]

Chronic Kidney Disease

In the past, small studies investigating the excretion of L-FABP in the setting of diabetic nephropathy have been mixed. Some have reported a link between decreased urinary concentrations of L-FABP in the setting of renin-angiotensin-aldosterone blockade and preserved GFR, whereas others found no correlation.[281,282] More recently, L-FABP has been investigated in established cohorts of CKD patients (e.g., ARIC, CRIC). In a case-control study of 321 patients from the ARIC cohort, 135 with ESRD, urinary L-FABP, normalized to urine creatinine, was not associated with an increased risk of ESRD.[253] These results are slightly different from when L-FABP was investigated in the CRIC cohort. Using Cox proportional hazard models, L-FABP was assessed in 2466 members of the cohort, with an average of a 3.8-year follow-up.[254] Although several quintiles were associated with progression of CKD (50% drop in eGFR or development of ESRD; fifth quintile unadjusted HR of 10.67 [7.46–15.25]), this effect was entirely attenuated when adjusting for factors known to affect the development and progression of CKD. As such, it appears that L-FABP is not an ideal biomarker for the detection or progression of CKD.

MONOCYTE CHEMOATTRACTANT PROTEIN-1

Monocyte chemoattractant protein-1 (MCP-1) is a chemotactic protein secreted by a variety of cells that attracts blood

monocytes and tissue macrophages through interaction with the cell surface receptor CCR2 (chemokine C-C motif receptor 2).[283,284] Induction of MCP-1 at the transcript or protein level has been demonstrated in a variety of human cell types, including fibroblasts, endothelial cells, peripheral blood mononuclear cells, and epithelial cells, on proinflammatory stimuli.[284–288] Kidney cells also produce MCP-1 in response to proinflammatory cytokines, including TNF-α and IL-1β.[289]

Acute Kidney Injury

Plasma MCP-1 was measured in 972 participants of the TRIBE AKI cohort to determine its association with postoperative AKI and mortality.[290] In this subset of TRIBE patients, 34% of whom had AKI ($n = 329$), MCP-1 levels were significantly higher in those who developed AKI or died (12%; $n = 119$) in the median 2.9-year follow-up period. Preoperative MCP-1 levels in the highest tertile were associated with a 43% increased risk of AKI compared with those in the lowest preoperative tertile; however, this effect was not significant when looking only at an outcome of severe AKI. This analysis was likely limited by the low number of patients who developed severe AKI (stage 2 or 3 AKI, $n = 45$; 5%). Those in the highest preoperative tertile were also at increased risk of death, with an adjusted HR of 1.95 (1.09–3.49).[290] Although this TRIBE study represents the largest study to date of the role of MCP-1 as a biomarker of AKI, others have investigated it in other settings, including DGF following kidney transplantation.[291] Given these limited data, as well as the strength of its role in CKD, we anticipate that MCP-1 will continue to be investigated as a biomarker of AKI over the next several years. Munshi and associates have demonstrated that urinary levels of both MCP-1 transcripts and protein were elevated in patients with AKI, as well as in experimental models of AKI; however, these data have yet to be replicated in other AKI cohorts.[292]

Chronic Kidney Disease

The expression of MCP-1 is induced in kidney diseases with significant inflammation, such as diabetic nephropathy and other glomerulonephropathies.[293–295] In particular, podocytes and tubular cells produce MCP-1 in response to high levels of glucose and advanced glycosylation end products.[296] Furthermore, urine levels of MCP-1 are significantly elevated in patients with diabetic nephropathy, and its levels correlate significantly with albuminuria and NAG levels in humans, as well as in experimental diabetic nephropathy.[297–300] In a prospective observational study of patients with diabetic nephropathy, urinary levels of connective tissue growth factor (CTGF) were elevated in patients with microalbuminuria and macroalbuminuria, but the urinary MCP-1 level was elevated only in those with macroalbuminuria.[301] Urinary CTGF levels correlated with progression to macroalbuminuria, whereas urinary MCP-1 levels (but not CTGF levels) correlated with the subsequent rate of eGFR decline (at a median follow-up of 6 years). The authors concluded that increased urinary CTGF concentration is associated with early progression of diabetic nephropathy, whereas MCP-1 level is associated with later-stage disease.[301] The independent association of urine MCP-1 with the risk of CKD progression has been confirmed by others.[302,303] Elevated levels of urinary MCP-1 were reported in patients with lupus nephritis, and the presence of MCP-1 in urine reflected its intrarenal expression.[304,305] Serum concentrations of MCP-1 were also shown to be elevated in patients with diabetic nephropathy and lupus nephritis, but the serum levels did not correlate with disease progression.[304–306] Moreover, the lack of correlation between urinary and serum MCP-1 levels suggests that urinary MCP-1 is the result of local production of MCP-1 by the kidney rather than simply filtration of serum MCP-1. More recently, Vianna and colleagues demonstrated that plasma and urinary levels of MCP-1 are elevated in pediatric CKD (from either glomerular disease or congenital anomalies) but were not correlated with each other. MCP-1 levels were significantly higher than in those without CKD, and there were differences in MCP-1 concentration, depending on the cause of the CKD.[295]

NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN

NGAL, also known as "lipocalin 2" or "lcn2," is one of the biomarkers of AKI that has been studied extensively. NGAL has many characteristics required for a good biomarker of AKI compared with serum creatinine measurement or urine output.[307] It is a 25-kDa protein with 178 amino acids belonging to the lipocalin superfamily. Lipocalins are extracellular proteins with diverse functions involving transport of hydrophilic substances through membranes, thereby maintaining cell homeostasis.[308] NGAL is a glycoprotein bound to matrix metalloproteinase-9 in human neutrophils. It is expressed in various tissues in the body, such as salivary glands, prostate, uterus, trachea, lung, stomach, and kidney,[309] and its expression is markedly induced in injured epithelial cells, including those in the kidney, colon, liver, and lung.

Transcriptome profiling studies in rodent models have identified NGAL as one of the most upregulated genes in the kidney very early after tubular injury.[310,311] Mishra and associates have demonstrated that NGAL levels are significantly elevated within 2 hours after injury in mouse models of renal ischemia–reperfusion.[312] In addition, urinary NGAL was detectable after 1 day of cisplatin administration, which suggests its sensitivity in other models of tubular injury.[312]

Acute Kidney Injury

Many clinical studies have followed these important observations in animals. Mishra and associates first demonstrated the value of NGAL as a clinical marker in a prospective study of 71 children undergoing CPB.[313] In this study, both serum and urinary NGAL levels were upregulated within 2 hours in patients who developed AKI. A cutoff value of 50 μg/L was 100% sensitive and 98% specific in predicting AKI. Following this seminal paper, several other papers investigating NGAL in the setting of cardiac surgery were published, with several demonstrating that both urine and serum NGAL levels were able to predict AKI earlier than serum creatinine, as well as correlate with AKI severity.*

However, no paper was able to replicate the near-perfect results published in this first study. Given the wealth of studies that have reported on NGAL over the past decade, we have chosen to highlight the larger and multicenter trials that studied NGAL.

*References 44, 45, 108, 109, 210, 211, 213, 242, 244, and 314–319.

Urine Neutrophil Gelatinase-Associated Lipocalin

In the setting of the ER, urine NGAL was originally shown to perform well in Nickolas and colleagues'[63] first study in 635 patients, demonstrating that a cutoff of 130 μg/g of creatinine had a sensitivity of 90% and specificity of 99.5% for AKI, defined as RIFLE risk.[320] In this single-center prospective study, urine NGAL also predicted the future need for nephrology consult, admission to the ICU, and need for RRT. In a follow-up multicenter study of 1635 subjects, urine NGAL had an AUC of 0.81 (0.76–0.86) for the prediction of AKI (RIFLE risk) and had an NRI of 26.1% while demonstrating the ability to improve the classification of both AKI events and nonevents. Additionally, urine NGAL values were significantly different and increased in a stepwise fashion in those with no AKI < CKD < prerenal AKI < intrinsic AKI.

Lieske and colleagues measured urine NGAL in 363 ER patients and determined that NGAL has an AUC of 0.70 for the detection of AKIN AKI while providing only modest sensitivity (65%) and specificity (65%).[320a] In addition to demonstrating that NGAL levels increased with the severity of AKI, they demonstrated that pyuria and urinary white blood cells were associated with increased urinary NGAL levels. Urine NGAL has been studied less extensively in the pediatric ER but has demonstrated similar potential in a smaller study ($n = 252$), with a lower AKI incidence rate ($n = 18$; 7.1%).[240]

Urine NGAL has also been studied in the setting of critical illness by Siew and colleagues, who demonstrated that urine NGAL was able to predict AKI within the first 24 (AUC = 0.71) and 48 (AUC = 0.64) hours of ICU admission.[321] In this single-center prospective study of 451 critically ill adults, urine NGAL was independently associated with the development of AKI, even after adjusting for factors known to be correlated with the development of AKI (including severity of illness and sepsis). NGAL performed similarly when measured in a post hoc analysis of the EARLY ARF trial. Data from this prospective observational study performed in two general (mixed) ICUs in New Zealand demonstrated that urine NGAL could modestly predict the development of AKI (AKIN stage 1; AUC, 0.66) but was also able to forecast the need for RRT (AUC, 0.79) and death within the first 7 ICU days (AUC 0.66; $P < .001$ for all three). Additionally, urine NGAL performed better in predicting AKI on ICU arrival in those with higher baseline eGFRs (AUC = 0.70 for those with an eGFR from 90–120 mL/min vs. an AUC of 0.64 for those with an eGFR < 60 mL/min).[216] This improved ability to detect the future development of AKI in those with a higher eGFR has been demonstrated by others in the setting of cardiac surgery.[44,45] In a separate post hoc analysis of the EARLY ARF study, urine NGAL values again demonstrated a significant increase across the spectrum of AKI, with concentrations being lowest in those without AKI and values for those with transient AKI that lasted less than 48 hours being in between those with no AKI and those with AKI that lasted longer than 48 hours.[217] In the discovery phase of the multicenter prospective observational SAPPHIRE trial, with 522 participants, urine NGAL had an AUC of 0.66 (95% CI, 0.60–0.71) for the development of RIFLE injury or failure within the first 36 hours of study enrollment; this increased to 0.71 (0.66–0.76) when looking at RIFLE injury or failure within the first 12 hours.[322]

In the setting of cardiac surgery, urine NGAL has provided similar mixed results. In the TRIBE-AKI adult cohort, the highest quintile of urine NGAL at 0 to 6 hours following surgery was associated with an increased risk of AKI (doubling of serum creatinine or need for RRT); however, this effect was no longer significant after adjusting for factors known to contribute to AKI risk.[210] In addition to having an AUC of 0.67 for the detection of AKI in this cohort of 1219 adults, urine NGAL levels were significantly associated with the composite endpoint of inpatient mortality or receipt of RRT, as well as length of ICU stay and length of hospitalization. All quintiles of urine NGAL were associated with duration of AKI in unadjusted analyses; however, this effect was no longer significant for all but the fourth quintile after adjusting for factors known to affect the development of AKI.[212] Urine NGAL did not display the ability to detect AKI progression in the 380 adults who developed at least AKIN stage 1. Although those subjects in the fifth quintile of urine NGAL at the time of serum creatinine increase were at increased risk for more progressive AKI (e.g., going from AKIN stage 1 to AKIN stage 3), this effect was no longer significant in the adjusted analysis.[213] These results were in contrast to those of the pediatric cohort ($n = 311$), in which those in the fifth quintile of urine NGAL remained at a significantly increased risk of developing AKI (doubling of serum creatinine or need for RRT), even after adjusting for the clinical model (OR 4.1; 95% CI, 1.0–16.3). Additionally, urine NGAL levels correlated with the length of mechanical ventilation, ICU stay, and hospitalization.[211]

In a separate secondary analysis looking at the long-term mortality of the adult TRIBE cohort in patients with AKI ($n = 407$,) those subjects in the third tertile of urine NGAL were at increased risk of death during the median 3.0-year follow-up. The adjusted HR for this group (compared with the first tertile) was 2.52 (95% CI, 1.86–3.42). A similar effect was not seen in the third tertile of those without AKI ($n = 792$), HR 0.90 (95% CI, 0.50–1.63).[214]

Urine NGAL has been investigated in the setting of DGF following renal transplantation, with several studies linking elevated urine NGAL levels in the posttransplantation setting with the development of DGF.[241,323] Reese and colleagues[257] shifted this paradigm slightly in measuring urine NGAL in the deceased donor urine rather than in the posttransplantation setting. They demonstrated that elevated urinary NGAL was associated the development of recipient DGF; the third tertile of NGAL had a relative risk of 1.21 (1.02–1.45) compared with the lowest tertile. Elevated deceased donor urine NGAL levels were weakly associated with slightly lower eGFR at 6 months posttransplantation in those patients who did not develop DGF. As such, although urine NGAL did correlate with donor-based AKI, it did not provide meaningful value in predicting short- and long-term outcomes posttransplantation.[257]

Urine NGAL has been investigated in several other smaller studies in more niche cohorts and has demonstrated some promise in detecting AKI and other adverse patient outcomes, including critically ill neonates,[324] individuals with cirrhosis or hepatorenal syndrome,[325,326] and 1-year graft survival following transplantation.[223] However, these trials and others require validation in larger and multicenter investigations.

Plasma Neutrophil Gelatinase-Associated Lipocalin

Plasma NGAL has been examined in many of the same studies with urine NGAL, including in the settings of the ER and ICU and following cardiac surgery. In the ER, Di Somma and colleagues[327] have demonstrated that the plasma NGAL drawn at the time of ER arrival had an AUC of 0.80 for the future development of AKI. In this multicenter prospective cohort study, the AUC improved to 0.90 when ER physician clinical judgment was added to plasma NGAL. This combination of physician clinical judgment and NGAL outperformed either physician judgment and serum creatinine alone, leading to a significant NRI of 32.4%.

In the setting of critical illness, plasma NGAL was measured as part of a post hoc analysis of the multicenter EARLY ARF study ($n = 528$), in which it had an AUC of 0.74 (0.69–0.79) for the development of AKIN stage 1 AKI ($n = 147$) during subsequent ICU stay. This study defined functional AKI based on the AKIN criteria but also defined structural AKI in terms of urine NGAL concentrations. Plasma NGAL performed even better (AUC = 0.79) at predicting urine NGAL–defined structural AKI ($n = 213$). In addition to strong associations with creatinine and urine NGAL–based definitions of AKI, plasma NGAL was also associated with the need for RRT ($n = 19$) but not with inpatient mortality ($n = 53$).[328] In the SAPPHIRE trial, which also examined the performance of plasma NGAL in the setting of ICU-associated AKI, NGAL had an AUC of 0.64 (0.58–0.70) for the detection of RIFLE injury or failure within the first 12 hours of study enrollment; this significant ability to forecast more severe forms of AKI did not dramatically change when assessing the ability to detect the same level of AKI over the first 36 hours (AUC, 0.64 [95% CI, 0.58–0.71]).[322]

It has been postulated that NGAL's performance in the setting of critical illness is likely attenuated in part due to the preponderance of sepsis-related AKI in the ICU, and that NGAL levels are inherently higher in those with sepsis because it is derived in part from neutrophils. De Geus and colleagues[39] published a prospective observational cohort study of 663 ICU admissions in which plasma NGAL levels were measured four times during the first 24 hours. They demonstrated that plasma NGAL levels were significantly higher in those with sepsis compared with those without sepsis, and that when the cohort was stratified according to the presence of sepsis ($n = 80$; 12% of the cohort), plasma NGAL was able to detect AKI remarkably well in both cohorts (AUC of 0.76 in those with sepsis vs. 0.78 in those without sepsis). These data corroborate the work of Katagiri and associates, which demonstrated in a prospective observational study of 139 critically ill patients that plasma NGAL levels are highest in those with sepsis associated with AKI > nonsepsis AKI > no AKI.[330] Future investigations of plasma NGAL will need to take this association with sepsis into account as we begin to construct normal ranges, as well as clinically validated cutoffs to use NGAL in interventional trials to treat those in the early stages of AKI.[331,332]

In addition to investigations in the setting of critical illness, plasma NGAL has been investigated in the setting of acute heart failure.[333] In a multicenter, prospective, observational cohort study of 927 patients with acute decompensated heart failure requiring intravenous diuretics, Maisel and colleagues evaluated plasma NGAL's ability to predict worsening of renal function, defined as a 0.5 mg/dL or ≥50% increase in serum creatinine level from baseline or the need for RRT.[333] In their study, 72 patients (7.8%) developed the primary endpoint with neither first or peak NGAL providing a significantly higher AUC compared with serum creatinine alone (0.656, 0.647, and 0.652 respectively). Similarly, plasma NGAL was predictive of in-hospital adverse events (AUC of 0.691) but did not significantly outperform serum creatinine (AUC, 0.686). This large-scale investigation does not support the use of plasma NGAL for the detection of AKI in the setting of decompensated heart failure.

Despite these disappointing findings in the setting of heart failure, plasma NGAL has been shown to predict recovery from AKI in preliminary studies. In 181 patients with community- acquired pneumonia and at least RIFLE failure AKI, plasma NGAL measures on the first day that met failure criteria were able to predict the failure of recovery of renal function. Individuals with high plasma NGAL levels were less likely to recover, with an AUC of 0.74. However, this was not significantly different from a clinical model consisting of age, serum creatinine level, and severity of illness scores.[334] This potential ability to detect nonrecovery of AKI is yet another aspect of NGAL and other biomarkers that require further investigation.

Plasma NGAL has also been extensively studied in the setting of cardiac surgery. In the TRIBE-AKI adult cohort ($n = 1219$), plasma NGAL levels were significantly higher in those who developed AKI (doubling of serum creatinine or RRT) in the early postoperative period. Those in the fifth quintile of plasma NGAL at the 0- to 6-hour time point (>293 ng/mL) were at a 7.8-fold increased risk of developing AKI compared with those in the first quintile (<105 pg/mL). This effect was attenuated after adjusting for factors known to associate with AKI but remained significant (OR, 5.0 [(95% CI, 1.6–15.3]), although it was no longer significant after adjusting for serum creatinine.[210] Additionally, plasma NGAL was significantly associated with increased length of ICU and hospital stay, as well as a composite of in-hospital death or dialysis. In this same adult cohort, plasma NGAL, measured at the time of a clinical AKI and serum creatinine increase, demonstrated a remarkable ability to detect those individuals with progressive AKI (e.g., going from AKIN stage 1 to stage 2 or 3; $n = 380$). After adjusting for the clinical model, the fifth quintile of plasma NGAL (>322 ng/mL) was nearly eight times more likely to develop progressive AKI (OR 7.72 [95% CI, 2.65–22.49]) compared with those in the first two quintiles. Plasma NGAL displayed the ability to improve the reclassification of both those with and without progressive AKI (events and nonevents), providing a category-free NRI of 0.69 ($P < .0001$).[213] The results from the TRIBE-AKI pediatric cohort were less promising, with plasma NGAL not displaying the ability to predict severe AKI (doubling of serum creatinine or need for RRT) in the early postoperative period. However, the fifth quintile of NGAL (>259 ng/mL) measured within the first 6 postoperative hours was significantly associated with the development of RIFLE risk AKI, with an adjusted OR of 2.3 (95% CI, 1.0–5.5),[211] although falling well short of the near-perfect performance published in the original Mishra paper.[335]

Outside of these large trials, there have been several smaller trials that investigated NGAL in a variety of AKI settings, so much so that Haase and colleagues conducted a pooled prospective study ($n = 2322$; 1452 with cardiac surgery and 870

with critical illness) that designated subjects as NGAL(+) or NGAL(–) and creatinine(+) or creatinine(–); creatinine (+) was defined as RIFLE-R.[41] After analyzing NGAL data from 10 separate prospective observational studies, they demonstrated that individuals who were NGAL(+) but creatinine(–) needed acute dialysis 16 times more often than those who were NGAL(–) creatinine(–) (OR, 16.4 [95% CI, 3.6–76.9]; $P < .001$). The study also demonstrated an incremental increase in ICU stay, hospital stay and mortality among the four study groups as follows: NGAL(–) creatinine(–) < NGAL(+) creatinine(–) < NGAL(–) creatinine(+) < NGAL(+) creatinine(+).

The function of NGAL as a diagnostic marker of contrast medium–induced nephropathy has also been evaluated. In a prospective study of 91 children undergoing coronary angiography, both urine and plasma NGAL levels were found to be significantly increased within 2 hours of contrast medium administration in the group that developed contrast-induced nephropathy but not in the control group. By comparison, AKI detection using increases in serum creatinine concentration was possible only 6 to 24 hours after contrast agent administration. When a cutoff value of 100 ng/mL was used, both urine and serum NGAL levels at 2 hours predicted contrast medium–induced nephropathy, with AUCs of 0.91 and 0.92, respectively.[336] In several studies of adults undergoing procedures requiring contrast, an early increase in both urine (4-hour) and plasma (2-hour) NGAL levels was documented, compared with a much later increase in plasma cystatin C levels; this provides support for the use of NGAL as an early biomarker for contrast medium–induced nephropathy.[224,337] A meta-analysis has revealed an overall AUC of 0.89 for the prediction of AKI when NGAL is measured within 6 hours after contrast agent administration when AKI is defined as a 25% or greater increase in serum creatinine concentration.[338]

The origin of plasma and urinary NGAL after AKI requires further clarification. Gene expression and transgenic animal studies have demonstrated an upregulation of NGAL in the distal nephron segments, specifically in the thick ascending limb of Henle and the collecting ducts; however, most of the injury in AKI occurs in the proximal tubules.[339,340] On the other hand, the source of plasma NGAL in AKI is not well defined. For example, in animal studies, direct ipsilateral renal vein sampling after unilateral ischemia indicates that NGAL synthesized in the kidney does not enter the circulation.[340] The increase in plasma NGAL observed in AKI may derive from the fact that NGAL is an acute-phase reactant and may be released from neutrophils, macrophages, and other immune cells. Yndestad and colleagues have reported strong immunostaining for NGAL in cardiomyocytes within the failing myocardium in studies of experimental and clinical heart failure.[341] Furthermore, any impairment in GFR resulting from AKI would be expected to decrease renal clearance of NGAL, with subsequent accumulation in the systemic circulation. However, the contribution of these mechanisms to the rise in plasma NGAL concentration after AKI has yet to be investigated.

NGAL levels are also influenced by various medical conditions, such as CKD, hypertension, anemia, systemic infections, hypoxia, inflammatory conditions, and cancer, which makes it relatively less specific for kidney injury.[342] Additionally, there is some evidence to suggest that NGAL concentrations degrade over time, with levels decreasing by nearly 50% within the first 6 months of storage at –80°C. These degradation issues also affect other biomarkers (including *N*-acetyl-β-D-glucosaminidase and KIM-1); their impact on clinical results remains unclear because this remains an area of continued investigation.[343] Nevertheless, NGAL represents a very promising candidate as a biomarker for the early diagnosis of AKI and potential prediction of outcome.

Chronic Kidney Disease

In addition to extensive investigation in the setting of AKI, NGAL has been increasingly investigated in the setting of CKD. Some of this work was inspired by animal data that demonstrated that NGAL, like KIM-1, was highly upregulated by the persistent inflammation and late immune response following AKI and potentially contributes to the development of post-AKI CKD.[248] Moving this concept into humans, Nickolas and colleagues have reported on the correlation of NGAL with histologic changes on native kidney biopsies of subjects with CKD. They demonstrated that NGAL levels were inversely correlated with eGFR and directly correlated with both interstitial fibrosis and tubular atrophy.[320]

Over the past several years, there have been several investigations into the ability of urine NGAL to detect incident and progressive CKD, using previously collected cohorts, such as ARIC, MESA, and CRIC. In an original case-control substudy of the ARIC cohort ($n = 286$), urinary NGAL did not initially correlate with baseline eGFR. However, the fourth quartile of urine NGAL was at more than a twofold increased risk of developing incident stage 3 CKD during the follow-up period. It should be noted, however, that this effect was attenuated after adjusting for urine creatinine and urine albumin.[252] In a subsequent investigation of ARIC participants, 135 patients with ESRD and 186 matched controls (gender, race, baseline renal function, and diabetes status), there was no association of urine NGAL (normalized to urine creatinine) with the development of ESRD over time.[253]

Analysis of data from the MESA cohort was unable to find an association between urine NGAL levels and the future development of incident CKD stage 3. In a 1:1 nested case-control study, NGAL levels were not associated with the development of CKD stage 3 or a decrease in eGFR of >3 mL/min per year over a 5-year follow-up period.[249]

Finally, several analyses from CRIC have investigated the role of urine NGAL in predicting CKD and its progression. Liu and colleagues have demonstrated that there is a strong association between baseline urine NGAL and the risk of CKD progression (defined as a 50% reduction of MDRD-calculated eGFR or development of ESRD) over the mean follow-up of 3.2 years.[344] However, although this effect was significant in an unadjusted analysis, urine NGAL offered no improved prediction after adjusting for baseline age, race, eGFR, proteinuria, diabetes, and other factors known to affect CKD progression (C-statistic of 0.847 for both). Thus, in this cohort of 3386 individuals, urine NGAL offered no improvement in predicting CKD outcomes compared with more traditional markers. More recently, in a subset of 2466 CRIC enrollees with slightly longer follow-up (mean, 3.8 years) Hsu and colleagues demonstrated that the higher four quintiles of urine NGAL (normalized to urine creatinine) were associated with CKD progression. However, after adjusting for factors known to affect CKD progression, this effect was no longer significant in all quintiles.[254]

These findings from ARIC, MESA, and CRIC, which demonstrate an association with CKD progression that does not persist in adjusted models, are in direct contrast to a recent prospective observational cohort of 158 white patients with baseline CKD stage 3 or 4. This study demonstrated that urine NGAL (adjusted for urine creatinine) was associated with CKD progression; 40 patients reached the primary endpoint of all-cause mortality or need for RRT during the 2-year follow-up. The baseline urine NGAL was associated with this composite primary endpoint, with every increase of urine NGAL of 5 μg/mmol being associated with a 27% increased risk of death or RRT.[345] These findings are similar to those of Bolignano and colleagues, who performed a prospective observational study of 96 subjects with CKD, with a median follow-up of 18.5 months.[346] They demonstrated that both urine and serum NGAL were associated with a composite endpoint of either doubling of baseline serum creatinine or the development of ESRD. Conversely, in a 4-year follow-up study of 78 patients with type 1 diabetes conducted to evaluate the potential of urinary NGAL level to predict progression to diabetic nephropathy, NGAL levels were not associated with decline in GFR or development of ESRD and death after adjustment for known progression promoters.[347]

N-ACETYL-β-D-GLUCOSAMINIDASE

NAG is a lysosomal brush border enzyme that resides in the microvilli of tubular epithelial cells. Damage to these cells results in shedding of this enzyme into the urine. NAG has a high molecular weight of 130 kDa, and hence plasma NAG is not filtered by the glomeruli. Its excretion into urine correlates with tubular lysosomal activity. Increased urinary concentrations of NAG have been found in patients with AKI, chronic glomerular disease, diabetic nephropathy, exposure to nephrotoxic drugs, delayed renal allograft function, environmental exposure, contrast medium–induced nephropathy, or sepsis and following CPB.[239,348–354] In a prospective study involving 201 hospitalized patients with AKI, patients with higher concentrations of urinary NAG and KIM-1 were more likely to die or require dialysis. This study suggests the utility of NAG in combination with KIM-1 in predicting adverse clinical outcomes in patients with AKI.[239] Urinary NAG concentrations were significantly higher in patients with contrast medium–induced nephropathy than in patients without such nephropathy within 24 hours after the administration of a contrast agent.[353]

Similarly, in a two-center Japanese study of 77 patients undergoing cardiac surgery, NAG levels were elevated in those who developed postoperative AKI.[265] In this study, biomarker performance significantly improved when NAG was combined with L-FABP (an AUC improvement from 0.75 to 0.81). This same group published a single-center study investigating the performance of NAG in predicting the development of AKI (RIFLE) in a mixed medical-surgical ICU. NAG did not perform as well in this cohort of 339 subjects, providing an AUC of 0.62 for the development of RIFLE risk.[218] In a cohort of 635 ER patients, a NAG value over 1.0 units/g had an AUC of 0.71 (95% CI, 0.62–0.81) for the development of AKI during the subsequent hospital admission. However, this effect was attenuated in a multivariable analysis that included other novel and traditional biomarkers of AKI (e.g., creatinine, BUN, NGAL).[176]

In the setting of CKD, a study of patients with type 1 diabetes and nephropathy, Vaidya and colleagues have shown that lower levels of urinary KIM-1 and NAG are associated with the regression of microalbuminuria.[259] Similarly, in a nested case-control study from the Diabetes Control and Compliance Trial, baseline NAG concentrations were shown to predict micro- and macroalbuminuria.[355]

NAG has been investigated for its ability to detect CKD progression in a few cohorts. In the CRIC cohort, NAG was associated with CKD progression in unadjusted analyses, with the fifth quintile having an HR of 15.16 (10.17–22.59).[254] However, after adjusting for age, gender, race, and albuminuria, this association was completely attenuated. Additionally, the ability of NAG to predict CKD progression was analyzed in 149 patients with chronic heart failure over 5-year follow-up.[250] Those with a urine NAG level above the median were nearly four times as likely (OR, 3.92; $P = .001$) to have their CKD progress (defined as ≥25% decrease in eGFR). NAG was also shown to be an independent predictor of all-cause mortality.[250]

There are some limitations in the use of NAG as a marker of kidney injury. Inhibition of NAG enzyme activity has been reported in the presence of metal ions and at higher urea concentrations in the urine. Moreover, increased urinary levels of NAG have been reported in several nonrenal diseases, including rheumatoid arthritis and hyperthyroidism, as well as in conditions with increased lysosomal activity without cellular damage.[356,357] Because of concerns about its specificity, the clinical utility of NAG as a biomarker has been limited.

PROTEINURIA

In a healthy person, urinary protein excretion is less than 150 mg/day and consists mainly of filtered plasma proteins (60%) and tubular Tamm-Horsfall protein (40%).[358,359] Proteinuria can result from at least three different pathophysiologic mechanisms, including glomerular (increased permeability of glomerular filtration barrier to protein due to glomerulopathy, raised glomerular capillary hydrostatic pressure, or altered glomerular filtration coefficient), overflow (due to increased production of low-molecular-weight plasma proteins, such as immunoglobulin light chains in myeloma), and tubular processes (decreased tubular absorption of filtered proteins or increased production of tubular proteins by damaged tubules). Proteinuria mechanisms and consequences are discussed in Chapter 30. Proteinuria is diagnosed when the total urinary protein is greater than 300 mg/24 hours. Methods for detecting and monitoring proteinuria are discussed in Chapter 23.

Some reports have highlighted the diagnostic power of total protein for AKI in various drug-induced nephrotoxicities, including cisplatin and nonsteroidal antiinflammatory drugs.[360,361] A low eGFR is a known risk factor for AKI, and the utility of proteinuria in combination with the eGFR to predict the risk of this disease is now being investigated.[362] In a large cohort of nearly 1 million adult Canadians, James and colleagues demonstrated an independent association among eGFR, proteinuria, and incidence of AKI.[363] This group reported that patients with normal eGFR levels (≥60 mL/min/1.73 m^2) and mild proteinuria (urine dipstick, trace to 1+) have 2.5 times more risk of admission to a hospital with AKI than patients with no proteinuria. The risk was increased to 4.4-fold when they included patients with heavy

proteinuria (urine dipstick ≥ 2+). Adjusted rates of admission with AKI and kidney injury requiring dialysis remained high in patients with heavy dipstick proteinuria, independent of the eGFR.[18] These findings confirm previous reports suggesting that the eGFR and proteinuria are potent risk factors for subsequent AKI.[364,365]

ALBUMINURIA

Albuminuria is recognized as one of the most important risk factors for the progression of CKDs. Albumin is a major serum protein with a size slightly larger than the pores of the glomerular filtration membrane, so albuminuria is best known as a biomarker of glomerular dysfunction; its appearance in large amounts in urine represents compromised integrity of the glomerular basement membrane.[366] In smaller amounts, however, the presence of albumin in the urine may reflect tubular injury. Albuminuria is classified in the KDIGO classification system as A1 (UAE < 30 mg/day or urine ACR [uACR] < 30 mg/g creatinine), A2 (previously termed "microalbuminuria"; urinary albumin excretion, 30–300 mg/day, or uACR, 30–300 mg/g creatinine) and A3 (previously termed "macroalbuminuria"; urinary albumin excretion > 300 mg/day or uACR > 300 mg/g creatinine). In a number of clinical studies, albuminuria has been shown to be a sensitive biomarker of drug-induced tubular injury.[367,368] It is routinely used as a marker of kidney damage for making a CKD diagnosis at eGFR levels above 60 mL/min/1.73 m^2.[359] Guidelines of the National Kidney Foundation (NKF) and American Heart Association (AHA) include microalbuminuria, as well as an increase in the urinary total protein excretion, as a risk factor for renal and cardiovascular disease. Both NKF and AHA guidelines suggest measurement of the uACR in an untimed spot urine sample. Ideally, the uACR should be assessed in at least three different samples to decrease intraindividual variation.[369] Albuminuria is a continuous risk factor for ESRD and cardiovascular mortality, with no lower limit, even after adjustment for the eGFR and other established risk factors.[370–372] Urinary albumin has been used as a biomarker for monitoring CKD progression and potential therapeutic efficacy, although the FDA does not accept albuminuria as a surrogate marker. Using microalbuminuria as a marker, Levin and colleagues have demonstrated that N-acetylcysteine may attenuate contrast-induced glomerular and tubular injury.[373]

In the past several years, there have been increased investigations of albuminuria as a biomarker for AKI. In a large-scale, collaborative meta-analysis, Grams and colleagues combined data from eight general population cohorts (1,285,049 subjects) and five CKD cohorts (79,519 subjects) to investigate the association of albuminuria and other factors with AKI.[374] The primary outcome for this study was hospitalization for AKI. Using Cox proportional hazards models, they demonstrated that the incidence of AKI was higher in those with CKD (2.6%) compared with the general population (1.3%). Additionally, compared with those with an uACR < 5 mg/g, the risk of AKI with uACR > 300 mg/g was 2.73 (2.18–3.43].[374] Thus, increased uACR is a strong risk factor for the long-term risk of developing of AKI.

The TRIBE AKI measured preoperative albuminuria in 1159 adult patients, organizing the cohort into clinical risk categories based on the preoperative uACR: 10 mg/g or less (≤1.1 mg/mmol), 11 to 29 mg/g (1.2–3.3 mg/mmol), 30 to 299 mg/g (3.4–33.8 mg/mmol), and 300 mg/g or greater (≥33.9 mg/mmol). The incidence of AKI, defined as AKIN stage 1, increased across the uACR categories with those in the >300 mg/g category having a relative risk (RR) of 2.36 (95% CI,1.85–2.82) compared with the <10 mg/g group. This association was slightly attenuated after adjusting for variables known to affect proteinuria and AKI, with a RR of 2.21 (95% CI, 1.66–73).[375] Similarly, the fourth and fifth quintiles of urine albumin (in mg/L) were associated with increased duration of AKI in unadjusted analyses (fifth quintile, OR 2.83 [1.94–4.12].[212] Although this effect disappeared for the fourth quintile after adjusting for factors known to affect the development of AKI, there was only slight attenuation of the fifth quintile (2.21 [1.48–3.30].[212] These adult data are in contrast to pediatric TRIBE-AKI data (n = 294), which demonstrated no association between the preoperative uACR and the development of postoperative AKI.[376] The adult uACR data represent an additional biomarker to aid in cardiac surgery AKI prediction models and supplement other recent data that point to proteinuria and albuminuria serving as biomarkers of AKI in the preoperative and postoperative setting.[377,378]

In the postoperative setting, the TRIBE-AKI cohort demonstrated that urinary albumin concentrations (mg/L) and dipstick proteinuria values within 6 hours of adult cardiac surgery correlated with the future development of AKI. Compared with the lowest quintile, the highest quintile of albuminuria (mg/L) and highest group of dipstick proteinuria were associated with the greatest risk of AKI (adjusted RR, 2.97 [95% CI, 1.20–6.91]), and adjusted RR of 2.46 (95% CI, 1.16–4.97), respectively]. However, only the postoperative urine albumin concentration (mg/L; not indexed to urine creatinine) was associated with improved risk stratification when added to the clinical model (AUC increased from 0.75 to 0.81; P = .006). Urinary albumin (mg/L) in the early postoperative period was also highly predictive of long-term mortality in the TRIBE AKI adult cohort. Specifically, in those with perioperative AKI (n = 407), those in the second tertile of albuminuria were at increased risk of death in the 3.0-year follow-up period (adjusted HR 2.28 [95% CI, 1.06–4.88]). Although this effect was further magnified in the third tertile of those with AKI (HR, 2.85 [95% CI, 1.36–5.99]), there was no increased mortality across any of the tertiles of urine albumin in the 792 subjects without perioperative AKI.[214] Despite its known utility in other settings, a higher early postoperative uACR (mg/g creatinine) was not statistically associated with AKI risk. The poor performance of uACR in the context of adult cardiac surgery may be explained by variations in the urine creatinine excretion within and between individuals, which could be especially prominent when renal function is not in a steady state, and may in part explain why urine albumin concentration (mg/L) outperformed uACR.

In the TRIBE AKI pediatric cohort, perioperative values of the uACR (mg/g), and not urine albumin concentration (mg/L), were found to be predictive of AKI. In children younger than 2 years, an absolute first postoperative uACR ≥ 908 mg/g (103 mg/mmol, highest tertile) predicted the development of AKIN stage 2 or 3 AKI, with an adjusted RR of 3.4 (95% CI, 1.2–9.4) when compared with the first tertile. In children 2 years of age or older, a postoperative uACR ≥ 169 mg/g (19.1 mg/mmol, highest tertile), regardless of

preoperative values, predicted stage 1 AKI after adjusting for clinical factors, such as age, race, gender, and preoperative eGFR, type of cardiac surgery, and adjusted RR of 2.1 (95% CI, 1.1–4.1).[376] Although urine albumin concentration and uACR remain established and readily available laboratory tests, the diversity of results when investigating the development of postoperative AKI indicates that further studies are needed before it may be used in clinical practice.

URINARY CYSTATIN C

Urinary cystatin C tracks the function of proximal tubular cells. In healthy individuals, the urinary levels of cystatin C are almost undetectable. Any damage to proximal tubular cells can impede the reabsorption and enhance the urinary excretion of cystatin C. Several clinical studies have sought to understand the potential of urinary cystatin C levels for the prediction of kidney injury and its prognosis. Herget-Rosenthal and associates analyzed data from 85 patients in the ICU who were at high risk of developing AKI and used the RIFLE classification to define AKI.[379,380] In their studies, the authors reported that the urine cystatin C level detected AKI 1 to 2 days before changes in serum creatinine level, with an AUC of 0.82 and 0.97 on day 2 and day 1, respectively, as well as demonstrating that urine cystatin C serves as a marker of AKI severity correlating with a future need for RRT. Urinary cystatin C-to-creatinine ratios of more than 11.3 mg/mmol were significantly associated with proteinuria. Attempts to validate urine cystatin C as a marker of ICU-associated AKI have provided mixed results. Siew and colleagues have measured urine cystatin C in 380 ICU patients (mixed surgical-medical, trauma, and cardiac) and demonstrated that there is no difference in concentrations between those with and without AKI ($P = .87$).[271] More encouraging data from the EARLY ARF trial have demonstrated that in a cohort of 529 subjects, urine cystatin C may have limited ability to detect AKI (AUC of 0.67 on ICU arrival), with no significant difference in its ability to detect AKI in those with a GFR above and below 60 mL/min.[216] Additionally, in a separate post hoc analysis of the same study, urine cystatin C values exhibited a stepwise and significant ($P < .001$) increase when comparing values from those with no AKI < prerenal AKI < intrinsic AKI (defined as AKIN stage 1 > 48 hours).

In comparison with these mixed ICU-associated AKI data, several small studies investigating urine cystatin C in the setting of cardiac surgery have demonstrated promise.[44,109,317,381] However, these results were not validated in the TRIBE-AKI cohort. In unadjusted analyses of the adult cohort, several quintiles of urine cystatin C were significantly associated with the development of both mild (AKIN stage 1) or severe (doubling of creatinine or need for RRT) AKI at both the 0- to 6-hour and 6- to 12-hour postoperative time points. However, the small associations were completely attenuated after adjusting for the clinical model. Similarly, in the TRIBE pediatric cohort, no quintile remained significantly associated with AKI (mild or severe) in the adjusted analyses.[382] Urine cystatin C demonstrated similar results when measured in a cohort of 1635 ER patients, supplying an AUC of 0.65 (95% CI, 0.58–0.72) for the future development of AKI. However, using a multivariable analysis that included traditional (creatinine) and more modern (urine NGAL, KIM-1, IL-18, and LFABP) biomarkers, urine cystatin C was not a significant contributor in predicting the composite outcome of inpatient RRT or death.[219] Finally, when investigated in a prospective multicenter observation cohort study of deceased donor kidney transplants, urine cystatin C concentration from the first postoperative day was modestly correlated with 3-month allograft function; the AUC for predicting DGF at the 6-hour postoperative time point was 0.69.[383]

A number of studies have reported increased urinary cystatin C levels in patients with proteinuria, which suggests the possibility of tubular damage as a consequence of protein overload.[384–386] Currently, urinary cystatin C level has several disadvantages as a biomarker, including lack of international standardization and expense of the assay. Although serum cystatin C has been demonstrated as a reliable biomarker of eGFR, one must remember that cystatin C synthesis is increased in smokers, patients with hyperthyroidism, those receiving glucocorticoid therapy, and those with elevated levels of inflammatory markers, such as white blood cell count and C-reactive protein level, and the impact of these factors on urine cystatin C in the setting of AKI has not been fully investigated.[387,388] Furthermore, several different commercial assays are available to measure cystatin C. Advantages are that the commercially available immunonephelometric assay provides rapid automated measurement of cystatin C, and results are available in minutes.[389] In addition, preanalytic factors, such as routine clinical storage conditions and freezing and thawing cycles, and interfering substances, such as bilirubin or triglycerides, do not affect cystatin C measurement.[389,390]

PROENKEPHALIN

Enkephalins are small endogenous opioid peptides; because they are known to be expressed in the kidney, they have been investigated for the ability to detect both AKI and CKD.[391] Physiologically, preproenkephalin is the primary gene product of the *PENK* gene, but this propeptide is eventually cleaved, and proenkephalin (pro-ENK)is generated in this process. Given its low molecular weight, it is freely filtered at the glomerulus and, as such, is potentially ideal as a biomarker of glomerular function. Plasma pro-ENK concentrations have been shown to be stable in vitro for 48 hours, unlike other enkephalins, which have proven more difficult to measure (e.g., met-enkephalin).[392]

Acute Kidney Injury

Pro-ENK has been investigated in a few AKI clinical settings, including a retrospective observational cohort of patients with suspected sepsis in the emergency department.[393] Given that it is freely filtered, plasma levels of pro-ENK were inversely correlated with creatinine clearance ($r = -0.72$). Additionally, pro-ENK levels were significantly higher in those who eventually developed AKI (defined by RIFLE criteria), compared with those without AKI. Pro-ENK on arrival in the ER had an AUC 0.815 ($P < .001$) for the future development of AKI. Additionally, pro-ENK was modestly associated with 7-day inpatient mortality, with an AUC of 0.69; this significantly outperformed serum creatinine for this endpoint (AUC, 0.61; $P = .045$).[393]

Pro-ENK has also been evaluated for its ability to detect AKI following adult cardiac surgery.[394] In a single-center cohort of 92 patients, 20 of whom developed postoperative AKI (AKIN definition), preoperative and early postoperative pro-ENK levels were higher in those who went on to develop AKI. Preoperative values provided a C-statistic of 0.68

($P = .013$); early postoperative values gave a C-statistic 0.72 ($P < .001$).[394]

In a separate investigation, pro-ENK levels were measured in 1908 patients with acute heart failure to investigate its relationship with renal function, as well as short- and long-term mortality.[395] Pro-ENK levels were independently associated with an increased risk of worsening of renal function ($n = 264$), defined as 0.3 mg/dL or 50% increase in serum creatinine from the admission value within the first 5 days. In an adjusted analysis, elevated pro-ENK levels on admission were associated with increased odds of developing a subsequent rise in serum creatinine (OR, 1.58 [1.25–2.00]; $P < .0005$). In this multicenter cohort of patients with cardiorenal syndrome and AKI, after multivariable Cox proportional hazards analyses, pro-ENK was independently associated with in-hospital and 1-year mortality. Finally, in the largest investigation of pro-ENK to date, pro-ENK was highly correlated with eGFR and plasma urea levels, with an F-statistic of 296 and 166, respectively ($P < .001$ for both).[395]

Chronic Kidney Disease

Although pro-ENK appears to be a useful biomarker in the setting of early AKI, its relationship with eGFR requires further evaluation, because limited data have suggested that it may also serve as a biomarker of CKD.[396] In a prospective cohort of 2568 patients without CKD (defined as eGFR > 60 mL/min/1.73 m^2), plasma pro-ENK was found to be associated with incident CKD. Those in the highest tertile of baseline pro-ENK level had an increased incidence of CKD compared with the lowest tertile (OR 1.51 [1.18 to 1.94]; $P < .001$). Additionally, pro-ENK improved the reclassification of patients for the detection of incident CKD in over 14% of the cohort. Additionally in this same cohort, there was a signal that a minor allele in the pro-ENK gene was also associated with an increased risk of CKD. Although much like the other associations, these need to be validated in separate cohorts.[396]

URINE TISSUE INHIBITOR METALLOPROTEINASE-2 AND INSULIN-LIKE GROWTH FACTOR–BINDING PROTEIN-7

Urine tissue inhibitor metalloproteinase-2 and insulin-like growth factor–binding protein-7 (TIMP-2 and IGFBP-7) have been shown to serve as biomarkers of AKI in the setting of critical illness. These biomarkers are unique in that they play a role in cell cycle arrest. Both IGFBP-7 (through p523 and p21) and TIMP-2 (through p27) block the effect of cyclin-dependent protein kinase complexes and cause short periods of G1 cell cycle arrest.[397–399] They were originally discovered as part of a three-center discovery cohort of 522 subjects These patients had AKI stemming from sepsis, shock, major surgery, and trauma. Over 300 potential markers were evaluated, with TIMP-2 and IGFBP-7 being the two that best predicted the development of KDIGO stage 2 or 3 AKI. This finding was then validated in a prospective international multicenter observational study of 728 subjects.[322] In this validation study, TIMP-2 and IGFBP-7 remained the top two performing biomarkers for the prediction of RIFLE injury or failure within the first 12 to 36 hours of study enrollment, with AUC values of 0.77 and 0.75, respectively (SAPPHIRE study). When these two biomarker values were multiplied together, they demonstrated an improved ability to detect this same endpoint (AUC of 0.80). The paper did not supply information about the combination of TIMP-2 and IGFBP-7 with other biomarkers of AKI (e.g., NGAL, KIM-1, L-FABP, IL-18).[322] However, these same biomarkers (TIMP-2 and IGFBP-7) were further validated in the OPAL study, where they provided a similar AUC for the detection of impending stage 2 or 3 AKI.[400] Perhaps more importantly, this study identified cut points for TIMP-2 × IGFBP-7 to help risk-stratify patients for severe AKI. Patients with a value less than 0.3 were at lowest risk for severe AKI, those with a value between 0.3 and 2.0 had a RR 4.7 (1.5–16.0) times higher, and those with a value greater than 2.0 had a RR 12.0 (4.2–40) times higher than the lowest risk group. These data cut points were further validated in a post hoc analysis of the original 728 SAPPHIRE cohort.[400]

This reproducibility of these results in distinct cohorts led the FDA to clear TIMP-2 × IGFBP-7 for clinical use in late 2014.[322,400,401] Additionally, Liu and colleagues have published data demonstrating that biomarker concentrations correlate to the clinical adjudication of a panel of three expert nephrology adjudicators who were blinded to the biomarker values.[402] Since that time, several post hoc investigations of these and other cohorts have demonstrated that these biomarkers have several strengths. In the 9-month follow-up of the SAPPHIRE cohort, TIMP-2 × IGFBP-7 levels measured at the time of study enrollment were shown to correlate with a composite endpoint of death or need for RRT.[403] There was a stepwise increase in risk of the endpoint across the biomarker cutoff strata (0.3 and 2.0) in those subjects who went on to develop AKI; those with a value greater than 2.0 had an adjusted HR of 2.16 [1.32–3.53] compared with those with values less than 0.3.[403] TIMP-2 × IGFBP-7 has also been shown to reliably forecast impending severe AKI in several subsets of critically ill patients, including those with CKD and congestive heart failure, as well as those undergoing emergent and cardiothoracic surgery.[404,405] Additionally, others have investigated these biomarkers in other clinical settings, such as pediatric AKI[406] and DGF following renal transplantation,[291] and further validation of these markers is required outside the setting of adult critical illness. Given the FDA and European clearance for clinical testing, the American Society of Nephrology's AKI Advisory group and others have published guidance documents on the potential clinical use of TIMP-2 × IGFBP-7 (Box 27.2).[407,408]

Furosemide Stress Test

Furosemide and other diuretics have long been used in the setting of AKI to convert oliguric AKI into nonoliguric AKI, but they have seen a resurgence for the ability to ascertain

Box 27.2 TIMP-2 × IGFBP-7

1. Urinary TIMP-2 × IGFBP-7 has been FDA-approved for determining which high-risk critically ill patients are at increased risk of severe AKI.
2. Elevated TIMP-2 × IGFBP-7 levels (>0.3) measured early in the course of an ICU stay can forecast the development of at least stage 2 AKI and have been shown to be associated with posthospitalization outcomes, such as 9-month mortality and the need for RRT.

tubular reserve in the setting of AKI.[409–411] As a loop diuretic, furosemide (an organic anion) is not filtered by the glomerulus because it is tightly bound to serum proteins (albumin), and it therefore gains access to the tubular urinary space through active secretion in the proximal convoluted tubule via the human organic anion transporter (hOAT) system.[412,413] In the tubular lumen, furosemide inhibits the ability of the thick ascending limb of the loop of Henle to transport chloride, preventing sodium reabsorption and resulting in natriuresis and increased urine flow and potentially reducing tubular oxygen demand.[414–418] As a result of these pharmacokinetic factors, the urine output following furosemide dosing has been hypothesized to represent an approach to assess the renal tubular integrity in the setting of AKI.

In 1973, Baek and colleagues studied 15 subjects who did not yet have clinically apparent AKI (as measured by changes in serum creatinine).[410] They subjected them to a one-time furosemide challenge assessing their UOP and free water clearance (C_{H_2O}). Although they did not standardize the furosemide dosing, or report if any of their patients had preexisting CKD, they did report that a C_{H_2O} near zero and a poor urine output response to furosemide signaled that "acute renal failure was imminent."[410]

More recently, Chawla and colleagues have modified and standardized this protocol, renaming it the "furosemide stress test" (FST).[419] They prospectively investigated the ability of the urinary response following the administration of the FST to predict adverse patient outcomes in patients with KDIGO stages 1 and 2 AKI.[39] In a cohort of 77 subjects, they reported that the 2-hour urine output response to 1.0 mg/kg FST (in the furosemide-naïve) and 1.5 mg/kg FST (in those with prior loop exposure) was able to forecast the progression to KDIGO stage 3 AKI ($n = 25$; 32.4%), with an ROC AUC (standard error) of 0.87 (0.05). A cutoff of 200 mL at 2 hours provided a sensitivity and specificity of 87.1% and 84.1%, respectively, for progression to stage 3 AKI. This same cutoff provided an AUC (SE) of 0.86 (0.08) for the receipt of inpatient RRT ($n = 11$; 14.2%) and 0.70 (0.09) for inpatient mortality ($n = 16$; 20.7%).[419]

In a secondary analysis of this same cohort, FST outperformed plasma and urine NGAL, urinary IL-18, and TIMP-2 and IGFBP-7 for prediction of progression to stage 3, need for RRT, and mortality.[420] Interestingly, when the FST data were analyzed in a subset of patients with elevated biomarkers (urine NGAL > 150 ng/mL; $n = 44$ or TIMP-2 × IGFBP-7 > 0.3; $n = 32$), its ability to detect these same outcomes was further improved. Thus, combining AKI biomarkers with a functional assessment of tubular reserve like the FST may be an informative bedside tool to assist clinicians in the prognostication of early AKI.[420] In this study, the FST was not performed in subjects deemed to be hypovolemic, those with obstructive uropathy, or those with a baseline eGFR less than 30 mL/min/1.73 m^2.[419]

Conversely, Van der Voort and colleagues[421] investigated the ability of a standardized infusion of furosemide to predict renal recovery in those with advanced AKI requiring RRT. In a post hoc analysis, they reported that urine output (median IQR) was higher in patients who recovered their renal function and eventually stopped RRT (654 mL [333–1155] vs. 48 [15–207] mL; $P = .007$). Their version of the FST for prediction of recovery had a diagnostic performance AUC ROC of 0.84. Regardless of the clinical scenario in which it is being tested, the urinary response to furosemide informs the clinician about the renal reserve present during AKI (progression or recovery). The FST provides a measure of proximal tubule secretory capacity but also assesses thick ascending limb and distal collecting duct function. Thus, the FST serves as an assessment of integrated nephron function and warrants further validation in larger and more diverse cohorts.

GENETIC ASSOCIATIONS WITH ACUTE KIDNEY INJURY RISK

In addition to investigating novel proteins and functional aspects of the nephron, others have focused their attention on the role that genetics play in predisposing patients to an increased risk of AKI and CKD. Discussions of the genetics of chronic kidney disease can be found in Section VI of this edition and are beyond the scope of this chapter. With regard to AKI, there have been several investigations over the past few years reporting associations with several polymorphisms.

Susantitaphong and colleagues have demonstrated that a polymorphism in the promoter region of the TNF-α gene was associated with AKI severity and distant organ dysfunction.[422] Using a cohort of 262 hospitalized adults, they demonstrated that after adjustment for race, gender, age, baseline eGFR, sepsis, and the need for RRT, those with the minor allele (rs1800629) had higher peak serum creatinine and higher multiple organ failure scores, compared with the GG genotype. In a cohort of 401 patients with acute lung injury enrolled in the Fluids and Catheter Treatment Trial (FACTT), Bhatraju and colleagues have reported that two minor alleles in the nuclear factor of kappa light-chain polypeptide gene enhancer in B cells inhibitor (NFKBIA) are strongly associated with the development of AKIN criteria for AKI. For rs1050851 and rs2233417, the ORs for any AKI were 2.34 (1.58–3.46; $P = 1.06 \times 10^{-5}$; false discovery rate (FDR) = 0.003) and 2.46 (1.61–3.76; $P = 1.81 \times 10^{-5}$; FDR = 0.003) for each minor allele, respectively.[423] The associations were increased for AKIN stages 2 and 3, with respective ORs of 4.00 (2.10–7.62; $P = 1.05 \times 10^{-5}$; FDR = 0.003) and 4.03 (2.09–7.77; $P = 1.88 \times 10^{-5}$; FDR = 0.003).

In one of the larger investigations around the genetic basis for postoperative AKI, Stafford-Smith and colleagues used a discovery cohort of 873 nonemergent coronary bypass patients and then attempted to replicate their findings in a 380-subject validation cohort.[424] Using linear regression, they adjusted for a clinical AKI risk score to test single-nucleotide polymorphism (SNP) association with peak postoperative serum creatinine level. In the discovery cohort, nine SNPs that met statistical significance were detected; two of these, rs13317787 in GRM7|LMCD1-AS1 intergenic region (3p21.6) and rs10262995 in BBS9 (7p14.3), were replicated with significance in the validation cohort. Further investigation of these two regions and meta-analyses found genome-wide significance at the GRM7|LMCD1-AS1 locus and a significantly strong association at BBS9.[424] These are two new loci that had not been previously described.

More recently, the largest investigation of the genetic susceptibility to in-hospital AKI was conducted by Parikh and colleagues.[425] They performed an exploratory genome-wide association study in 760 cases of AKI and 669 controls (hospitalized patients without AKI) and attempted to validate their findings in an additional 206 cases of AKI and 1406

controls. They assessed 609,508 SNPs and, in their replication analyses, they validated four SNPs with an increased odds of association with the development of AKI. These SNPs included rs62341639 (OR, 0.64 [0.55–0.76]; $P = 2.48 \times 10^{-7}$) and rs62341657 (0.65 [0.55–0.76]; $P = 3.26 \times 10^{-7}$) on chromosome 4 near the APOL1-regulator IRF2, and rs9617814 (0.70 [0.60–0.81]; $P = 3.81 \times 10^{-6}$) and rs10854554 (0.67 [0.57–0.79] $P = 6.53 \times 10^{-7}$) on chromosome 22.[425]

Prior to these more recent larger studies, a handful of systematic reviews of the genetic predisposition to AKI were published.[426–428] They concluded that despite several gene polymorphisms with documented associations with the development, severity, and outcome of AKI, definitive conclusions would require more investigation, with replication in new cohort studies.[427] We anticipate further investigation of the role of genetics in AKI risk in the years to come.

CHRONIC KIDNEY DISEASE BIOMARKERS

Currently, the eGFR and proteinuria are used as markers of CKD progression because of their widespread availability and ease of performing the tests. Because all forms of CKD are associated with tubulointerstitial injury, markers of tubular injury, including KIM-1, IL-18, NGAL, and L-FABP, have been investigated in CKD and shown to associate with outcomes in CKD due to a variety of causes, as discussed previously. In addition, elevated systemic levels of molecules that have impaired kidney clearance or increased production in CKD (e.g., asymmetric dimethylarginine, fibroblast growth factor 23) as well as chemokines (e.g., monocyte chemoattractant protein-1) and fibrotic markers (e.g., connective tissue growth factor, TGF-β1, and collagen IV) are discussed here. For more in-depth discussion around specific markers for glomerular diseases, cystic diseases, diabetes, and inherited forms of renal disease, please see the individual chapters covering these topics.

Table 27.7 summarizes the ability of biomarkers to detect clinical endpoints related to CKD in a variety of clinical settings.

PLASMA ASYMMETRIC DIMETHYLARGININE

Nitric oxide is synthesized by oxidation of the terminal guanidine nitrogen of L-arginine by nitric oxide synthase (NOS). This process can be reversibly inhibited by guanidine-substituted analogs of L-arginine, such as in asymmetric dimethylarginine (ADMA).[429,430] Three types of methylated arginines have been described in vivo—ADMA, N^G-monomethyl-L-arginine, and symmetric dimethylarginine, an inert isomer of ADMA. Of these, ADMA is the major type of endogenously generated methylated arginine that displays inhibitor activity of NOS. However, administration of ADMA to endothelial NOS knockout mice also induces vascular lesions, which suggests that ADMA may have actions independent of nitric oxide and NOS in vivo.[431] Vallance and associates first reported that plasma levels of ADMA are elevated in patients with renal failure[432] and hypothesized that impaired renal clearance of ADMA may account for the rise in plasma levels. This assumption has been challenged by follow-up studies in animal models demonstrating that only a small portion of circulating ADMA is excreted in the urine.[433] Moreover, elevated plasma ADMA levels are also reported in patients with incipient renal disease but normal renal function.[434]

Elevated plasma levels of ADMA have been reported in patients with a variety of cardiovascular risk factors, such as hypertension, diabetes, and hyperlipidemia.[435–437] Among these groups, plasma ADMA levels are particularly high in patients with CKD, patients with ESRD undergoing hemodialysis or peritoneal dialysis, and kidney transplant recipients.[432,438,439] Plasma levels of ADMA are strongly associated with carotid intima to media thickness, left ventricular hypertrophy, cardiovascular complications, and mortality in patients with ESRD.[440–442] Plasma ADMA levels have been shown to correlate prospectively, in an inverse manner, with CKD progression in those with and without diabetes-related CKD.[443,444] Similarly, ADMA levels may also be associated with the presence of proteinuria and albuminuria.[445]

Table 27.7 Biomarker Performance in Detecting Chronic Kidney Disease (CKD): Multicenter Studies

Parameter	Diagnosis of CKD	Progression of CKD to ESRD	Cardiovascular and Mortality Risk Assessment in CKD	Progression of HIV-Associated CKD
Urine NGAL	+	–	?	?
Blood NGAL	+	–	?	?
Blood CysC	+	+	+	+
Urine IL-18	–	?	?	+
Urine KIM-1	+	+	?	–
Plasma KIM-1	+	+	?	?
β-Trace protein	–	?	+	?
Urine protein and albumin	+	+	+	+
FGF23	–	+	+	?
TNFR1, R2	+	+	?	?
suPAR	+	+	?	?
EGF	+	+	?	?

CysC, Cystatin C; *EGF,* epidermal growth factor; *ESRD,* end-stage renal disease; *FGF23,* fibroblast growth factor 23; *IL-18,* interleukin-18; *KIM-1,* kidney injury molecule-1; *NGAL,* neutrophil gelatinase-associated lipocalin; *suPAR,* soluble urokinase-type plasminogen activator receptor; *TNFR,* tumor necrosis factor receptor; *+,* data published display the ability to detect this aspect of CKD; *–,* data published do not display the ability to detect this aspect of CKD; *?,* no large multicenter data published on this biomarker or aspect of CKD.

More recently, larger longitudinal studies have been performed linking ADMA with several forms of CKD and CKD outcomes. In a nested case-control study performed in the Genetics of Diabetes Audit and Research Tayside Study (GO-DARTS), ADMA and several other biomarkers were measured to determine their association with CKD progression in patients with type 2 diabetes.[446] In this study, progression of CKD was defined by a 40% or greater reduction in baseline eGFR over the 3.5-year study period, whereas in controls the eGFR decreased by less than 5% over this same period. ADMA levels were strongly associated with CKD progression in an analysis adjusted for clinical variables known to affect CKD progression, as well as several other biomarkers. Higher ADMA levels were associated with more rapid progression of CKD; for each standard deviation change in ADMA levels there was an increased adjusted odds of 8.36 (3.83–20.4; $P < .001$. This effect was larger than any of the other 14 biomarkers that they measured in this cohort, including KIM-1 (1.93 [1.18–3.27]; $P = .011$) and H-FABP (0.63 [0.38–1.02]; $P = .06$).[446] In a prospective observational cohort of 1157 patients (663 with diabetic kidney disease, 273 with glomerulonephritis, and 221 with cystic and/or interstitial disease), ADMA levels were measured to determine their association with mortality prior to the initiation of RRT. ADMA levels were associated with an increased risk of mortality in both the diabetic cohort (HR, 1.3 [1.1–1.6]) and the glomerulonephritis cohort (HR, 1.5 [1.3–1.8]).[447] Despite these emerging data, larger longitudinal studies are needed to demonstrate the ability of ADMA to identify CKD and predict its progression in cohorts with CKD due to multiple causes.

FIBROBLAST GROWTH FACTOR 23

Fibroblast growth factor 23 (FGF23) is a 32-kDa protein consisting of 251 amino acids coded by the FGF gene located on chromosome 12 in the human genome. FGF23 serves as a endocrine hormone that is secreted by osteoblasts and osteoclasts, which binds a FGF receptor and its coreceptor Klotho and stimulates phosphaturia.[448–450] In addition to promoting phosphaturia, FGF23 also decreases levels of 1,25-dihydroxy vitamin D, as well as parathyroid hormone (PTH).[451,452] Several cross-sectional studies have demonstrated that FGF23 levels are elevated in both adult and pediatric CKD populations.[453–455] In a prospective observational study of 3879 subjects in CRIC, FGF23 levels were shown to be increased in individuals with CKD stages 2 to 4, with FGF23 elevation occurring in the absence of abnormalities in serum phosphate and PTH.[456] As such, FGF23 has emerged as a candidate biomarker to detect CKD and abnormalities of bone mineral metabolism in the absence of changes in serum phosphate and PTH.

Apart from bone, FGF23 is principally expressed in the ventrolateral thalamic nucleus in mice and is also known be secreted in minimal amounts in the liver, heart, thymus, and lymph node. Maintenance of phosphate homeostasis is carried out by the sodium-dependent phosphate cotransporters NaPi-IIa and NaPi-IIc at the brush border membrane of proximal tubule cells in kidney, and FGF23 has been shown to regulate the activity of these transporters.[457,458]

FGF23 is increased in CKD and is a prognostic indicator for cardiovascular disease in patients with CKD.[459] In a recent analysis of 3860 participants of CRIC, FGF23 levels in the highest quartiles (compared with the lowest) were independently associated with a graded risk of congestive heart failure (HR, 2.98 [95% CI 1.97–4.52]) and atherosclerotic events 1.76 (95% CI, 1.20–2.59), even after adjustment for eGFR, proteinuria, and other traditional cardiovascular risk factors.[460] Other studies have shown that elevated plasma FGF23 concentrations are associated with cardiovascular events in patients not requiring dialysis, as well as with mortality in patients receiving hemodialysis, with levels in those with ESRD reaching nearly 1000-fold above normal.[461] Interestingly, FGF23 levels have also been shown to decline rapidly in individuals with prompt allograft function in the setting of renal transplantation.[462,463] It has been reported that serum FGF23 concentrations may be a useful marker for predicting the future development of refractory hyperparathyroidism and the response to vitamin D therapy in patients receiving dialysis.[464] Similarly, FGF23 may be useful in the evaluation of calcium, phosphate, and vitamin D disorders in early-stage CKD in pediatric as well as adult patients.[465,466] Lowering FGF23 levels (e.g., with oral phosphate binders) may reduce cardiovascular morbidity in CKD patients,[467] although further studies are required to investigate this further. For further discussion of FGF23, see Chapter 53.

SOLUBLE UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR

Although there has long been a connection between soluble urokinase-type plasminogen activator receptor (suPAR) and CKD in the setting of focal segmental glomerulosclerosis (FSGS) and other glomerular disease, this biomarker has been associated more broadly with CKD. For more on the role of suPAR in the setting of FSGS and other glomerulonephritides, see Chapter 31. suPAR is the circulating form of a glycosyl-phosphatidylinositol–anchored, three-domain membrane protein that is expressed on a variety of cells, including endothelial cells and podocytes.[468] There are circulating and bound forms of this protein, with the circulating form being produced through the cleavage of the membrane-bound form, thus making it detectable in several body fluids, including urine.[468,469] It has been proposed that soluble urokinase-type plasminogen activator receptor (suPAR) contributes to the pathogenesis of kidney disease in the setting of FGSS as well as diabetic nephropathy through the prevention of podocyte migration and apoptosis.[470,471] Although many of the specifics of these mechanisms remain an area of intense investigation, testing of human samples for suPAR has begun to yield interesting results.

Recently, Hayek and colleagues published a seminal paper measuring plasma suPAR levels in 3683 patients enrolled in the Emory Cardiovascular Biobank.[472] They then determined baseline and follow-up renal function in 2292 of these patients to determine the relationship between suPAR and baseline GFR, change in GFR, and development of CKD (defined as eGFR < 60 mL/min/1.73 m^2). Higher baseline suPAR levels were associated with a greater decline in eGFR/year. Patients in the highest quartile of baseline suPAR lost over four times as much eGFR/year compared with those in the lowest quartile (−4.2 mL/min compared with −0.9 mL/min). Similarly, the risk of progressing to an eGFR < 60 mL/min over the study period was 3.13 (2.11–4.65]) in those participants in the highest suPAR quartile compared with the lowest quartile ($n = 1335$ total patients who started with an eGFR > 60 mL/min). Although suPAR appears to be independently associated

with the development and progression of CKD, further studies are needed to validate these findings.

URINARY RENAL FIBROSIS MARKERS

Excessive production of extracellular matrix (collagen IV) and profibrotic growth factors including CTGF and TGF-β1 have been implicated in the progression of renal fibrosis. Several reviews on the ability of renal fibrosis markers to predict patient outcomes have been published.[473]

CONNECTIVE TISSUE GROWTH FACTOR

CTGF (also known as "CCN2"), a member of CCN family of matricellular proteins, was first discovered by Bradham and colleagues in 1991 as a secreted protein in the conditioned media of human umbilical vascular endothelial cells.[474] CTGF has been implicated in a variety of cellular functions, including proliferation, cell adhesion, angiogenesis, and wound healing.[440,475,476] Accumulated evidence on CTFG in the past few years has indicated that CTGF is both a marker and a mediator of tissue fibrosis.[477] CTGF is an immediate early gene potently induced by TGF-β and shown to promote fibrosis primarily through TGF-β.[478] CTGF is overexpressed in several fibrotic diseases, such as scleroderma and lung and hepatic fibrosis.[479–481] In the kidney, CTGF expression has been shown to be upregulated in various forms of renal disease, including IgA nephropathy, focal and segmental glomerulosclerosis, and diabetic nephropathy.[480] CTGF has been found to be elevated in the glomeruli at early and late stages of diabetic nephropathy.[482] Riser and associates first reported that CTGF is elevated in the urine of diabetic rats and in persons with diabetes.[483] Subsequently, several groups have reported higher urinary levels of CTGF in persons with diabetes than in healthy individuals,[484,485] which indicates its potential as a marker for diabetic nephropathy. In persons with diabetes, plasma CTGF levels were shown to be higher in those with macroalbuminuria than in those with a normal urine albumin level. CTGF was an independent predictor of ESRD and correlated with the rate of decline in the GFR.[486] In another study, both blood and urine levels of intact CTGF and the N-terminal fragment were measured in 1050 patients with type 1 diabetes from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complication (DCCT/EDIC).[487] Patients with macroalbuminuria had higher plasma levels of CTGF N-terminal fragment than those with or without microalbuminuria. Intact CTGF levels were associated with the duration of diabetes, as well as common carotid artery intima media thickness. Additionally, in regression analyses, log plasma CTGF N-terminal fragment concentrations were independently associated with the intima thickness of the common and internal carotid artery. Plasma CTGF concentration therefore serves as a risk marker for diabetic renal and vascular diseases.

More recently, urinary CTGF has been investigated for its ability to detect renal allograft fibrosis in a cohort of 315 transplant recipients.[488] Following this cohort for 2 years, Metalidis and colleagues compared CTGF concentrations with protocol biopsies at 3, 12, and 24 months posttransplantation. Urinary CTGF levels were independently associated with the degree of interstitial fibrosis.[488] In a subset of patients, CTGF levels at 3 months were associated with long-term moderate and severe fibrosis at the 24-month time point.

TRANSFORMING GROWTH FACTOR-β_1

TGF-β is essential for the development and differentiation of various tissues.[489] Three isoforms of TGF-β have been identified in mammalian species—TGF-β_1, TGF-β_2, and TGF-β_3. TGF-β_1 is the predominant isoform in humans.[490] It is mainly secreted as a high-molecular- weight inactive complex and undergoes a cleavage process for its activation.[491] Several studies have demonstrated the association of urine levels of TGF-β_1 with the progression of CKD. Elevated urinary TGF-β_1 levels were found in patients with glomerulonephritis and diabetic nephropathy, as well as in renal allograft recipients.[491–494] In addition, some of the profibrotic molecules induced by TGF-β_1, including TGF-β–inducible gene H3 (*βig-H3*) and plasminogen activator inhibitor-1 (PAI-1), were also detected at high levels in the urine.[495,496] Because TGF-β_1 is mostly secreted as an inactive complex that requires chemical modification for its activation, βig-H3 and PAI-1 can be used as surrogate markers for TGF-β_1 activity. Urinary levels of both *βig-H3* and PAI-1 have been shown to correlate with renal injury and fibrosis in patients with diabetic nephropathy.[495,496] However, in a study of 3939 participants from the CRIC study, TGF-β levels were not shown to be significantly associated with CKD progression or the presence of macroalbuminuria.[497] This is in comparison with a much smaller case-control study looking at TGF-β in the setting of pediatric obstructive nephropathy (posterior urethral valves), in which TGF-β levels were shown to be inversely correlated with the GFR.[498]

In the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) collaborative group, the addition of serum TGF-β to traditional predictors of CKD progression (e.g., diabetes, uACR, baseline eGFR) improved the prediction of doubling of serum creatinine over a 5-year follow-up period.[499] In this nested case-control study, addition of serum TGF-β significantly increased the AUC from 0.75 to 0.82 ($P < .0001$). Other more recent studies have demonstrated that urinary TGF-β can assist in determining which kidneys will recover function following unilateral obstruction.[500] In a small study of 45 subjects (11 of whom did not regain function), TGF-β levels at the time of enrollment were lower in those who went on to recover function.

COLLAGEN IV

Collagen IV is a component of the extracellular matrix, and excess deposition of collagen IV is present in renal fibrosis. Furthermore, elevation of urinary collagen IV has been reported in patients with IgA nephropathy, as well as in those with diabetic nephropathy, and has been correlated with declining renal function.[501,502] In a prospective observational cohort study of 231 normoalbuminuric and microalbuminuric patients with type 1 diabetes, urinary collagen IV was significantly associated with a decline in the eGFR over time in both univariate and multivariate analyses, with collagen IV levels being elevated in those with the lower GFRs.[503]

TUMOR NECROSIS FACTOR RECEPTORS

TNF is produced by immune cells and can either be membrane-bound or in a soluble circulating peptide.[504] TNF cell surface receptors have two forms (TNFR1 and TNFR2), both of which can be cleaved from the membrane with a TNF-α cleaving enzyme. TNFR1 is expressed in glomeruli

and endothelial cells, and TNFR2 is variably expressed in renal cells.[505] Increasingly, both receptors have been associated with adverse outcomes in the setting of kidney disease.[262,506]

Pavkov and colleagues performed a longitudinal cohort study in Native Americans with type 2 diabetes, following them for a median duration of 9.5 years.[506] Of a total of 193 participants, 62 developed ESRD and 25 died during the follow-up period. In an age- and gender-adjusted analysis, the highest quartile of TNFR1 and TNFR2 (compared with the lowest) was associated with an increased risk of ESRD, OR of 6.6 (3.3–13.3) and 8.8 (4.3–18.0), respectively. In more complex adjusted models (including both ACR and baseline GFR) the highest quartiles of TNFR1 and TNFR2 were associated with a 60% and 70% increased risk for ESRD compared with the lowest.[506]

More recently, Coca and colleagues measured TGFR1 and TGFR2 in subjects from the Action to Control Cardiovascular Risk in Diabetes (ACCORD) and Veterans Administration NEPHROpathy iN Diabetes (VA-NEPHRON-D) cohorts.[262] They demonstrated that receptor levels were independently associated with increased decline in GFR in persons with diabetic kidney disease. In the ACCORD cohort, which enrolled subjects with early diabetic kidney disease, those who developed incident CKD had higher TNFR1 and TNFR2 levels at the time of study enrollment. Similarly, in adjusted analyses, the fourth quartiles of TNFR1 and TNFR2 (compared with lowest quartile) were both associated with increased risk of incident CKD (OR, 95 CI%, 3.0 [1.2–7.3] and 8.4 [3.0–23.4], respectively).[262] In the NEPHRON-D study, the fourth quartiles of TNFR1 and TNFR2 (compared with the lowest quartile) were again associated with the primary endpoint, CKD progression. The highest quartile of TNFR1 was associated with an adjusted odds of 3.5 (1.9–6.3) for the progression of CKD, and this effect was even larger for TNFR2 (3.8 [2.0–7.3]).[262] TNFR1 and TNFR2 were highly correlated with each other in these two cohorts (Pearson correlation coefficient > 0.75). Thus, in several cohorts with diabetic kidney disease, TNFR1 and TNFR2 levels were associated with the development and progression of CKD.

EPIDERMAL GROWTH FACTOR

As a well-established growth factor, EGF has been shown to alter the kidney's response to tubulointerstitial injury,[507] with exogenous EGF improving outcomes in the setting of animal models of AKI.[508] However, in a recent investigation of CKD progression using a renal biopsy transcriptome-driven approach, EGF was shown to be an excellent biomarker of CKD progression, defined as the composite of a 40% reduction from baseline eGFR or the development of ESRD.[509] Intrarenal transcripts of EGF expression were correlated with baseline eGFR and patient outcomes in 164 patients from the European Renal cDNA Bank (ERCB). In this cohort, intrarenal EGF correlated with several other measures and outcomes, including urinary EGF, interstitial fibrosis and tubular atrophy, and eGFR loss. The authors were able to replicate these results in two separate CKD cohorts ($n = 55$ and $n = 48$; different samples from the ERCB cohort and Clinical Phenotyping Resource and Biobank Core [C-PROBE]).[509] Adding urine EGF to a model containing age, gender, baseline eGFR, and albuminuria significantly improved the C-statistic for the prediction of CKD progression from 0.75 to 0.87. This seminal report on EGF will be followed up with future investigations that will attempt to link this novel biomarker further with the development and incidence of progressive CKD.

COMBINATIONS OF MULTIPLE BIOMARKERS

In the classic biomarker paradigm, one biomarker detects one disease. However, acute and chronic kidney diseases are complex, with multiple underlying causes. A single biomarker may not be optimal to make an early diagnosis and predict the longer-term outcome of the disease process. Different biomarkers provide different sets of information. As discussed above, some biomarkers excel at detecting AKI in different clinical settings (cardiac surgery vs. ICU vs. ER vs. other), whereas others detect different aspects of AKI (early diagnosis vs. AKI severity vs. prerenal). This same phenomenon is true of CKD biomarkers, with some being more likely to detect CKD in the setting of diabetes versus obstruction versus other forms of glomerulonephritis. Thus, it is important to consider the clinical utility of a panel of biomarkers for acute and chronic kidney diseases.

It is becoming increasingly clear, based in part on the evidence discussed above, that multiple biomarkers are already a viable option in the care of patients with CKD. The serum creatinine level can be used in conjunction with cystatin C for the detection and diagnosis of CKD. Proteinuria and albuminuria can be used in combination with these two markers of glomerular function to diagnose and risk-stratify individuals further. Recall that in the setting of normal serum creatinine and serum cystatin C levels, the presence of albuminuria constitutes a diagnosis of CKD.

The use of multiple biomarkers in the setting of AKI has been an area of increased investigation, with several studies attempting to combine two or more biomarkers to improve their predictive capabilities.[242,243,265,322,330] Although some studies have simply used the product of two biomarkers and then assessed the AUC, others have used logistic regression models to assess the AUC for two or more biomarkers. There has not been a consensus for the statistical methods for combining biomarkers, and this remains an area of continued investigation. More recent studies have acknowledged the aforementioned premise that individual biomarkers will have their own specific kinetics, and that combining biomarkers from different time points may improve their predictive capabilities.[243] However, the clinical implications and feasibility of collecting biomarker samples at several distinct time points following cardiac surgery or ICU admission remain untested. As more and more biomarker data are amassed, we anticipate advances in novel methods for assessing biomarker combinations. Box 27.3 summarizes a variety of rationales and approaches to combining biomarker results in the hope of achieving improved prediction of patient outcomes.

CRITICAL PATH INITIATIVE: A NEED FOR BETTER BIOMARKERS

The Critical Path Initiative was launched in March 2004 by the FDA as a strategy for modernizing the sciences through which FDA-regulated products are developed, evaluated, manufactured, and used. In 2006, the Critical Path Initiative

Box 27.3 Strategies for Biomarker Combination

- Combine for different functions:
  - Combine a marker of filtration with one of tubular injury.
  - Combine a marker of proximal tubular injury with a marker of distal tubular injury.
- Combine for kinetics:
  - Combine biomarkers with different time courses to improve the duration of diagnosis.
- Combine for improved accuracy:
  - Use two or more biomarkers in statistical equations.
- Strategic combinations:
  - Combine a diagnostic biomarker with a prognostic biomarker.
  - Combine an extremely sensitive marker with an extremely specific marker.

outlined specific key areas of critical path focus identified by FDA experts and the public. Since that time, the FDA has provided guidelines stating that a biomarker can be considered "valid" only if (1) it is measured in an analytic test system, with well-established performance characteristics, and (2) there is an established scientific framework or body of evidence that elucidates the physiologic, pharmacologic, toxicologic, or clinical significance of the test result. Over the past decade there have been a few advancements in the development and approval of biomarkers of AKI and CKD.

In 2010, seven urinary proteins (KIM-1, albumin, total protein, β_2-microglobulin, cystatin C, clusterin, and trefoil factor 3) were evaluated for their utility to outperform current tests, including serum creatinine concentration and BUN concentration, in the detection of drug-induced kidney injury. The FDA and EMA qualified this panel of biomarkers for use in regulatory decision making for drug safety to detect acute drug-induced kidney injury in preclinical studies and, on a case-by-case basis, in early clinical studies in combination with standard biomarkers.[24,25,510,511] More recently, the FDA has approved total kidney volume in the setting of polycystic kidney disease as a marker of disease progression.[22] Given the aforementioned work investigating several promising biomarkers of AKI and CKD, there will undoubtedly be additionally measures approved in the next several years.

KIDNEY HEALTH INITIATIVE

In response to the epidemic of CKD and AKI and the limited number of randomized controlled trials, the FDA, American Society of Nephrology, and their industry partners announced the founding of the Kidney Health Initiative (KHI) in 2013. This public-private partnership was designed to foster collaborations to optimize the evaluation of drugs, devices, biologics and food products in the greater kidney community. This initiative is intended to facilitate the delivery of these products to the U.S. market in a safe and expeditious manner.[512] Despite the growing evidence of their ability to predict AKI, CKD progression, and other adverse patient outcomes, no new biomarkers have been approved in the United States for clinical use; as such, it remains to be seen how this new initiative will affect the biomarker field.

FUTURE OF BIOMARKERS

Recent advances in molecular analysis and proteomics have resulted in the identification of a wide range of potential serum and urine biomarkers for assessing renal function and injury, as well as predicting the development of kidney disease. Not only are many of these biomarkers sensitive, but some are also site-specific. A number of them have been reported to be predictive of an adverse outcome. For some, a great deal of additional work is still needed, however, to bring the biomarkers successfully to clinical practice.

Because kidney disease is complex, with multiple causes, and often presents in the setting of systemic diseases, a single biomarker may be insufficient for early diagnosis, insight into pathophysiology, and prediction of clinical course and outcome. Different biomarkers will be useful in different contexts. In some circumstances, a single biomarker may suffice, but in others, benefit will come from the use of multiple biomarkers in plasma, urine, or both to provide early evidence of risk and injury, and to distinguish between various types of kidney diseases. Many of these biomarkers can be grouped according to their association with a particular type of injury (e.g., podocyte or tubular injury) or mechanism of damage (e.g., oxidative stress, inflammation, fibrosis). Understanding the relationships between these different biomarker categories may help us better understand disease processes.

These biomarkers are not only useful for accessing kidney injury in humans in its early stages and for predicting progression of disease, but also crucial for translating novel therapeutic compounds from preclinical animal models to first human trials. Until recently, the use of newly emerged biomarkers in preclinical and clinical studies and drug development has been hindered by lack of regulatory acceptance. Hopefully, in the future, biomarker measurements obtained using biomarker test panels will not only be used to diagnose kidney injury and predict outcome, but will also be used as surrogate endpoints in clinical trials, which might speed up clinical evaluation of desperately needed therapies for kidney diseases.

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BOARD REVIEW QUESTIONS

1. The ideal biomarker for AKI should
   a. Differentiate between intrarenal, prerenal, and postrenal causes of acute kidney injury (AKI)
   b. Detect AKI early in the course and be able to predict the course of disease progression
   c. Be site-specific and able to correlate with histologic findings
   d. Be easily and reliably measured in a minimally invasive manner
   e. All of the above

   Answer: e

   Rationale: All of the above—the ideal marker for AKI should do all of the above.

2. Creatinine is an imperfect biomarker for AKI because of all the following, except
   a. Its production varies greatly with age
   b. Its production varies with gender
   c. It is specific for renal tubular injury
   d. Static measurements of serum creatinine do not reflect real-time changes in glomerular filtration
   e. Its assay is subject to interference from hyperbilirubinemia and elevated creatine kinase (CK) levels

   Answer: c

   Rationale: Creatinine is not specific for renal tubular injury. For example, intravascular volume depletion and prerenal factors (severe dehydration, blood volume loss, altered vasomotor tone, or age-related decrease in renal blood flow) and postrenal factors (obstruction or extravasation of urine into the peritoneal cavity) may falsely elevate serum concentrations in the absence of parenchymal damage. Thus, a decrease in the estimated glomerular filtration rate (eGFR) inferred from an increase in the serum creatinine level may not distinguish among prerenal, intrinsic renal, and postrenal causes of impaired kidney function, which may not be the case for some biomarkers of renal tubular injury.

3. As a biomarker of chronic kidney disease (CKD), all the following are true about serum cystatin C, except
   a. Cystatin C has been shown to be affected by gender
   b. Cystatin C is lower in older adults
   c. Cystatin C–based eGFRs improve CKD classification and risk stratification
   d. Cystatin C values are associated with mortality

   Answer: b

   Rationale: Unlike serum creatinine, which is usually lower in older adults, given their decreased muscle mass, a study investigating a subset of over 7500 subjects from the NHANES III study demonstrated that cystatin C levels were elevated in more than 50% of those older than 80 years.[91] Cystatin C levels have been shown to be associated with factors similar to those that are associated with creatinine—namely, these levels may be elevated in males, taller and heavier patients, and those with a higher lean body mass.[89–91] In a prospective cohort study of 26,643 Americans enrolled in the Reasons for Geographic and Racial Differences in Stroke (REGARDS. study), Peralta and colleagues have demonstrated that cystatin C–based eGFRs improve chronic kidney disease (CKD) classification and definition, as well risk stratification (for the development of end-stage renal disease and death, relative to creatinine-based eGFRs).[92]

4. Neutrophil gelatinase associated lipocalin (NGAL) is a
   a. 13-kDa, low-molecular-weight cysteine protease inhibitor produced at a constant rate by all nucleated cells and eliminated exclusively by glomerular filtration
   b. 18-kDa proinflammatory cytokine that is activated by caspase 1 and is produced by renal tubule cells and macrophages
   c. Type I transmembrane glycoprotein with an ectodomain containing a six-cysteine immunoglobulin-like domain, two *N*-glycosylation sites, and a mucin domain
   d. 25-kDa protein glycoprotein bound to matrix metalloproteinase-9 in renal epithelial cells
   e. Protein that selectively binds to free fatty acids and transports them to mitochondria or peroxisomes, where free fatty acids are beta-oxidized and participate in intracellular fatty acid homeostasis

   Answer: d

   Rationale: NGAL is a 25-kDa protein glycoprotein bound to matrix metalloproteinase-9 in renal epithelial cells. Cystatin C is a low-molecular-weight protein produced at a constant rate by all nucleated cells and eliminated exclusively by glomerular filtration. Interleukin 18 (IL-18) is an 18-kDa proinflammatory cytokine that is activated by caspase 1 and produced by renal tubule cells and macrophages. Kidney injury molecule-1 (KIM-1) is a type I transmembrane glycoprotein with an ectodomain containing a six-cysteine immunoglobulin-like domain, two *N*-glycosylation sites, and a mucin domain. Liver fatty acid binding protein (L-FABP) binds selectively to free fatty acids and transports them to mitochondria or peroxisomes, where free fatty acids are beta-oxidized and participate in intracellular fatty acid homeostasis.

5. A 74-year-old black woman with a history of CKD (baseline creatinine, 1.4 mg/dL; eGFR, 43 mL/min/1.73 m^2) and congestive heart failure (CHF), with a reduced left ventricular ejection fraction (20%), has undergone a combined a single-vessel coronary artery bypass graft (CABG) and mitral valve replacement. She had several minutes of documented hypotension in the operating room, which required transient use of norepinephrine, and had her aorta cross-clamped for 80 minutes, with a total cardiopulmonary bypass time of 120 minutes. On postoperative day 1, her creatinine was 1.9 mg/dL. The primary team decides to check the tissue inhibitor metalloproteinase-2 (TIMP-2) × insulin-like growth factor binding protein-7 (IGFBP-7) level, which returns a value of 1.2, and call a nephrology consult. Which of the following is true about the performance of this test in this patient?
   a. There is no increased risk for the development of AKI
   b. There is no increased risk for a composite endpoint of death or the need for dialysis in the next 9 months
   c. Her baseline CKD has no impact on the test results
   d. Her significant preoperative CHF (ejection fraction 20%) makes the test results difficult to interpret
   e. All of the above are true

Answer: c

Rationale: Her baseline CKD has no impact on the test results. Based on data from the SAPPHIRE and OPAL trials, TIMP-2 × IGFBP-7 can help risk-stratify patients for severe AKI. Patients with a value less than 0.3 were at lowest risk for severe AKI; those with a value between 0.3 and 2.0 had a relative risk 4.7 (1.5–16.0) times higher. In the SAPPHIRE study, TIMP-2 × IGFBP-7 levels measured at the time of study enrollment were also shown to correlate with a composite endpoint of death or need for renal replacement therapy over the next 9 months. In subgroup analyses, TIMP-2 × IGFBP-7 has also been shown to forecast impending severe AKI reliably in several subsets of critically ill patients, including those with advanced CKD, CHF, as well as those undergoing emergent and cardiothoracic surgery. As such, answer choice c is correct.

28 Pathophysiology of Acute Kidney Injury

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CHAPTER OUTLINE

PATHOPHYSIOLOGY OF CLINICAL ACUTE KIDNEY INJURY

The three major pathophysiologic categories—namely, prerenal, intrinsic, and postrenal (obstructive)—provide a framework for understanding the mechanisms of acute kidney injury (AKI).

PRERENAL ACUTE KIDNEY INJURY

Prerenal azotemia is the most common cause of AKI, accounting for approximately 40% to 55% of all cases.[1–3] It results from kidney hypoperfusion due to reductions in actual or effective arterial blood volume (EABV—the volume of blood effectively perfusing the body organs). Common conditions causing true hypovolemia include hemorrhage (traumatic, gastrointestinal, surgical), gastrointestinal (GI) losses (vomiting, diarrhea, nasogastric suction), renal losses (overdiuresis, diabetes insipidus), and third spacing (pancreatitis, hypoalbuminemia). In addition, cardiogenic shock, septic shock, cirrhosis, hypoalbuminemia, and anaphylaxis all are pathophysiologic conditions that decrease EABV, independent of total body volume status, resulting in reduced renal blood flow. Prerenal azotemia reverses rapidly if renal perfusion is restored because, by definition, the integrity of the renal parenchyma has remained intact. However, severe and prolonged hypoperfusion may result in tissue ischemia, leading to acute tubular necrosis (ATN). Therefore, prerenal azotemia and ischemic ATN are part of a spectrum of manifestations of renal hypoperfusion.

Prerenal azotemia has also been divided into volume-responsive and volume-nonresponsive forms. The former is easy to comprehend, but the latter is less straightforward. In volume-nonresponsive forms, additional intravenous volume is of no help in restoring kidney perfusion and function. Disease processes such as congestive heart failure, liver failure, and sepsis may not respond to intravenous fluids because markedly reduced cardiac output or total vascular resistance, respectively, prevent improved kidney function.

True or effective hypovolemia causes a decrease in mean arterial pressure that activates baroreceptors and initiates a cascade of neural and humoral responses, leading to activation of the sympathetic nervous system and increased production of catecholamines, especially norepinephrine. There is increased release of antidiuretic hormone, mediated primarily by hypovolemia, resulting in vasoconstriction, water retention, and urea back diffusion into the papillary interstitium. In response to volume depletion or states of decreased EABV, there is increased intrarenal angiotensin II (Ang II) activity via activation of the renin-angiotensin-aldosterone system (RAAS). Ang II is a very potent vasoconstrictor that preferentially increases efferent arteriolar resistance, preserving glomerular filtration rate (GFR) in the setting of decreased renal perfusion through the maintenance of glomerular hydrostatic pressure. In addition, Ang II increases proximal tubular sodium absorption through a combination of alterations in hydrostatic forces in the peritubular capillaries and through direct activation of sodium-hydrogen exchangers. During severe volume depletion, Ang II activity is even greater, leading to afferent arteriolar constriction, which reduces renal plasma flow, GFR, and the filtration fraction, and markedly augments proximal tubular sodium reabsorption in an effort to restore plasma volume.[4] Ang II has also been shown to have direct effects on transport in the proximal tubule through receptors located in the proximal tubule. It has also been postulated that the proximal tubule can produce Ang II locally. Hence, under conditions of volume depletion, Ang II stimulates a larger fraction of tubular transport, whereas volume expansion will blunt this response.[5–9]

Renal sympathetic nerve activity is significantly increased in prerenal azotemia. Studies have shown that in the setting of hypovolemia, adrenergic activity independently constricts the afferent arteriole and changes the efferent arteriolar resistance through Ang II. α_1-Adrenergic activity primarily influences kidney vascular resistance, whereas renal nerve activity is linked to renin release through β-adrenergic receptors on renin-containing cells. In contrast, α_2-adrenergic agonists primarily decrease the glomerular ultrafiltration

coefficient via Ang II. Although vasodilation might be expected as a result of acute removal of adrenergic activity, a transient increase in Ang II is actually seen, maintaining GFR and renal blood flow. Even after subacute renal denervation, renal vascular sensitivity increases to Ang II as a result of major upregulation of Ang II receptors. Hence complex effects on renin-angiotensin activity occur within the kidney secondary to increased renal adrenergic activity during prerenal azotemia.[10]

All these systems work together and stimulate vasoconstriction in musculocutaneous and splanchnic circulations, inhibit salt loss through sweat, and stimulate thirst, thereby causing retention of salt and water to maintain blood pressure and preserve cardiac output and cerebral perfusion. Concomitantly, there are various compensatory mechanisms to preserve glomerular perfusion.[11] Autoregulation is achieved by stretch receptors in afferent arterioles that cause vasodilation in response to reduced perfusion pressure. Under physiologic conditions, autoregulation works only to a mean systemic arterial blood pressure of 75 to 80 mm Hg. Below this level, the glomerular ultrafiltration pressure and GFR decline abruptly. Renal production of prostaglandins, kallikrein, and kinins, as well as nitric oxide (NO), is increased, contributing to the vasodilation.[12,13] Nonsteroidal antiinflammatory drugs (NSAIDs), by inhibiting prostaglandin production, worsen kidney perfusion in patients with hypoperfusion. Selective efferent arteriolar constriction, a result of Ang II, helps preserve the intraglomerular pressure and hence the GFR. Angiotensin-converting enzyme (ACE) inhibitors inhibit synthesis of Ang II and therefore disturb this delicate balance in patients with severe reductions in EABV, such as severe congestive heart failure or bilateral renal artery stenosis and, in these settings, can worsen prerenal azotemia. On the other hand, very high levels of Ang II, as seen in circulatory shock, cause constriction of both afferent and efferent arterioles, negating its protective effect.

Although these compensatory mechanisms minimize the progression toward AKI, they too are overcome in states of severe hypoperfusion. Renovascular disease, hypertensive nephrosclerosis, diabetic nephropathy, and older age predispose patients to prerenal azotemia[14] at lesser degrees of hypotension.[14] Prerenal azotemia also predisposes patients to radiocontrast media–induced AKI and events such as anesthesia and surgery, which are known to result in further decreases in renal blood flow. Therefore it is imperative to diagnose prerenal azotemia promptly and initiate effective treatment because it is a potentially reversible condition that can lead to ischemic ATN and/or nephrotoxic AKI if therapy is delayed, or the severity of the condition increases. In patients with advanced liver disease and portal hypertension, the hepatorenal syndrome (HRS) represents an extreme form of prerenal disease, characterized by peripheral and splanchnic vasodilation, with intense intrarenal vasoconstriction unresponsive to volume resuscitation.[15–17] AKI can also result from abdominal compartment syndrome (ACS), characterized by a marked elevation in intraabdominal pressure, resulting in a clinical presentation with features similar to those of prerenal AKI.[18,19] A recent study in animal models of volume-responsive and intrinsic AKI used laser microdissection to isolate specific domains of the kidney, followed by RNA sequencing of the microdissected kidney tissue. Based on the gene expression profile, the investigators found different signal transduction pathways in the two models. Volume-responsive genes rapidly reverse with volume resuscitation, whereas intrinsic AKI genes did not change. These results suggest that volume-dependent AKI is not an attenuated form of intrinsic AKI.[20]

INTRINSIC ACUTE KIDNEY INJURY

DISEASES OF LARGE VESSELS AND MICROVASCULATURE

Total occlusion of the renal artery or vein is an uncommon event but can be seen in certain scenarios such as trauma, instrumentation, thromboemboli, thrombosis, and dissection of an aortic aneurysm. Stenosis of the renal artery is a slow chronic process, with or without evidence of declining GFR, and rarely presents as an acute event. Renal vein thrombosis has classically and frequently been associated with hypercoagulable states, including nephrotic syndrome, particularly when associated with membranous nephropathy. An atheroembolic source should be considered in patients who present with AKI after instrumentation with angiography, arteriography, or aortic surgery or after blunt trauma or acceleration-deceleration injury.[21] Cholesterol-laden atheroembolic plaques in the aorta or other larger arteries may become disrupted, and fragments may become trapped in smaller renal arteries, leading to hypoperfusion and an intense inflammatory reaction, akin to a vasculitis. Other organs may also be affected, leading to gastrointestinal ischemia, peripheral gangrene, livedo reticularis, and acute pancreatitis. Patients frequently develop fevers and exhibit eosinophilia, an elevated erythrocyte sedimentation rate, and hypocomplementemia, which sometimes help in differentiating this condition from other simultaneous insults (e.g., transient hypotension and/or radiocontrast media administration).

Renal artery thrombosis is usually a posttraumatic or postsurgical complication, especially in the transplantation setting, but can also occur in other hypercoagulable states, such as antiphospholipid antibody syndrome.[22–24] Diseases affecting the small vessels, generally termed *vasculitides*, include polyarteritis nodosa, necrotizing granulomatous vasculitis, hemolytic-uremic syndrome, thrombotic thrombocytopenic purpura, and malignant hypertension; they tend to occlude vessels by fibrin deposition, along with platelets. Endothelial cell damage leads to an inflammatory response in the renal microvasculature (and in other organs), leading to reduced microvascular blood flow and tissue ischemia, sometimes giving rise to superimposed ATN. One should keep in mind the intricate relationship among these inflammatory vasculitides and subsequent ischemic injury because even though the origin of these disease processes is located at a site distant from the tubules, the final result is often ATN if not treated early. Hence, virtually any disease that compromises blood flow within the renal microvasculature can induce AKI.

DISEASES OF THE TUBULOINTERSTITIUM

Ischemic and septic ATN are the most common causes of intrinsic AKI. These are discussed extensively in later sections of the chapter on ATN. Other disorders of the tubulointerstitium causing AKI, such as acute allergic interstitial nephritis, drug-induced tubular toxicity, and endogenous toxins, are presented in the following sections.

Interstitial Disease

Acute interstitial nephritis (AIN) results from an idiosyncratic allergic response to different pharmacologic agents, most commonly to antibiotics (e.g., methicillin and other penicillins, cephalosporins, sulfonamides, quinolones), NSAIDs (e.g., ibuprofen, naproxen),[25] chemotherapeutic agents,[26] and proton pump inhibitors..[27] Although evidence supports a role of vancomycin as a tubule toxin, most biopsy reports have described AIN.[28] The expanding list of newer targeted agents has led to more cases of acute tubulointerstitial injury. Serine-threonine protein kinase inhibitors such as vemurafenib and dabrafenib target a protein called B-Raf and have been associated with acute and chronic tubulointerstitial damage.[29] Other agents that cause acute tubulointerstitial injury include checkpoint inhibitors such as ipilimumab, nivolumab, and pembrolizumab. Programmed cell death protein 1 (PD-1), a checkpoint protein, is expressed on T cells, and PD-L1 is expressed on normal as well as cancer cells. Binding of PD-1 ligand to its receptor (PD-L1) blocks immune elimination by T cells. Cancer cells evade immune surveillance in part by this mechanism. Similarly, CTLA-4 is another checkpoint protein expressed on T cells that leads to self-tolerance and various forms of autoimmune injury, including acute interstitial injury.[30]

Other conditions such as leukemia, lymphoma, sarcoidosis, bacterial infections (e.g., *Escherichia coli*), and viral infections (e.g., cytomegalovirus) can also cause AIN, leading to AKI. Systemic allergic signs such as fever, rash, and eosinophilia are often present in antibiotic-associated AIN but are not usually present in NSAID-related AIN, in which lymphocytes tend to predominate.[31] The presence of inflammatory infiltrates within the interstitium is the key hallmark of AIN. These inflammatory infiltrates are often patchy and present most commonly in the deep cortex and outer medulla. Interstitial edema is typically seen with the infiltrates, and sometimes patchy tubular necrosis may be present in close proximity to areas with extensive inflammatory infiltrates.[25] The composition of cells in the interstitial infiltrate can suggest the cause of AIN. The presence of frequent eosinophils suggests a diagnosis of drug-induced AIN, whereas a high number of neutrophils favors bacterial infection, and a high number of plasma cells is dominant in immunoglobulin G4 (IgG4)–related tubulointerstitial nephritis and in renal allografts infected with polyomavirus.[32] Most cases of AIN are probably induced by extrarenal antigens being produced by drugs or infectious agents that may be able to induce AIN by the following: (1) binding to kidney structures; (2) modifying immunogenetics of native renal proteins; (3) mimicking renal antigens; or (4) precipitating as immune complexes and hence serving as the site of antibody- or cellular-mediated injury.[33] This reaction is triggered by many events, including activation of complement and release of inflammatory cytokines by T cells and phagocytes. In other cases, loss of tolerance from checkpoint inhibitors leads to immune-mediated inflammation and injury.[26]

Tubular Disease—Exogenous Nephrotoxins

Nephrotoxic ATN is the second most common cause of intrinsic AKI. We shall briefly review the common drug nephrotoxicities in the context of AKI. The kidneys are vulnerable to toxicity due to the high blood flow, and they are the major elimination and metabolizing routes of many of these nephrotoxins. Furthermore, because of the medullary tonicity, the concentration of drugs within the tubular lumen increases along the nephron, exposing the tubules to toxic levels for a more prolonged exposure time. Several other well-known therapeutic agents, such as amphotericin B, vancomycin, acyclovir, indinavir, cidofovir, foscarnet, pentamidine, and ifosfamide, can all directly cause acute tubular injury and associated AKI.

Radiocontrast Media–Induced Nephropathy. Iodinated radiocontrast medium–induced nephropathy (CIN) is a common complication of radiologic or angiographic procedures. The incidence varies from 3% to 7% in patients without any risk factors but can be as high as 50% in patients with moderate to advanced chronic kidney disease (CKD). Other risk factors include diabetes mellitus, intravascular volume depletion, use of high-osmolality contrast media, advanced age, proteinuria, and anemia.[34,35]

Unlike many other forms of intrinsic tubular injury, radiocontrast medium–induced AKI is usually associated with urinary sodium retention and a fractional excretion of sodium (FE_{Na}) of less than 1%. AKI resulting from iodinated contrast media is typically nonoliguric and rarely requires dialysis. However, requirements for renal support, prolonged hospitalization, and increased mortality are associated with (although not necessarily caused by) this condition.

The pathophysiology of CIN likely consists of combined hypoxic and toxic renal tubular damage associated with renal endothelial dysfunction and altered microcirculation.[36,37] The administration of radiocontrast media causes vasoconstriction and markedly affects renal parenchymal oxygenation, especially in the outer medulla, as documented in various studies where the cortical Po_2 declined from 40 to 25 mm Hg and the medullary Po_2 fell from 26 to 30 mm Hg to 9 to 15 mm Hg.[37–39] Radiocontrast media injection leads to an abrupt but transient increase in renal plasma flow, GFR, and urinary output.[40] This effect is due to the hyperosmolar radiocontrast medium–enhancing solute delivery to the distal nephron and leads to increased oxygen consumption by enhanced tubular sodium reabsorption. Using video microscopy, it has also been documented that radiocontrast media markedly reduce inner medullary papillary blood flow, even to the extent of near-cessation of red blood cell (RBC) movement in papillary vessels, associated with RBC aggregation within the papillary vasa recta.[41] In isolated vasa recta from rats and humans, contrast medium applied to the lumen has led to constriction and enhanced vasa recta responses to Ang II.[42,43] However, it should be noted that there may be different patterns of response possibly related to the type, volume, and route of radiocontrast medium administration. Numerous neurohumoral mediators may contribute to the changes in renal microcirculation caused by radiocontrast medium injection. Intrarenal NO synthase activity, NO concentration, plasma endothelin, adenosine, prostaglandins, and vasopressin are all thought to play a role in altering the cortical and medullary microcirculation after radiocontrast medium injection. Mechanical factors may also play a role because radiocontrast media increase blood viscosity and may affect the flow in the complex, low-pressure medullary microcirculation.[39] An increased plasma viscosity after radiocontrast medium administration can interfere with blood flow, particularly under the hypertonic conditions of the

(inner) renal medulla, where the plasma viscosity is already increased as a result of hemoconcentration. Indeed, there are several animal studies that have shown a correlation between experimental CIN and viscosity of the radiopaque compound.[44,45]

Evidence has also suggested direct tubular toxicity from radiocontrast media. Early studies on isolated renal tubules in vitro have shown direct toxic effects of radiocontrast media on proximal tubular cells (PTCs).[46] Radiocontrast media (e.g., diatrizoate, iopamidol) induced a decline in tubule K^+, adenosine triphosphate (ATP), and total adenine nucleotide contents. At the same time, there was a decrease in the respiratory rate of the tubules and an increase in Ca^{2+} content. These changes were more pronounced with the very high-osmolality ionic compound diatrizoate than with the lower osmolality nonionic iopamidol. Importantly, the cytotoxic effects were aggravated by hypoxia, indicating interactions between direct cellular mechanisms and vasoconstriction-mediated hypoxia.[47] Andersen and coworkers have demonstrated the concentration-dependent radiocontrast media–mediated release of tubular marker enzymes, ultrastructural changes, and cell death in both Madin-Darby canine kidney (MDCK) and porcine kidney proximal tubule epithelial (LLC-PK_1) cells.[48] Radiocontrast medium–induced critical medullary hypoxia may lead to the formation of reactive oxygen species (ROS), with subsequent membrane and DNA damage. A vicious cycle of hypoxia, free radical formation, and further hypoxic injury may be activated after radiocontrast medium exposure. Clinically, CIN presents with an acute decline in the GFR within 24 to 48 hours of administration, with a peak serum creatinine concentration usually occurring in 3 to 5 days and return to baseline within 1 week, although patients with moderate to advanced CKD may take somewhat longer to return to the baseline serum creatinine concentration. Existing CKD, diabetic nephropathy, advanced age, congestive heart failure, volume depletion, and coincident use of NSAIDs also increase the risk for CIN.

Unlike the first-generation contrast media, high-osmolar contrast media (HOCM; osmolality of 1500–1800 mOsm/kg), low-osmolar contrast media (LOCM; osmolality of 600–850 mOsm/kg), and isosmolar contrast media (IOCM; osmolality of 270–320 mOsm/kg) are associated with a lower incidence of contrast-induced AKI.[49–51] However, whether the incidence of contrast-induced AKI is lower in IOCM than LOCM is still debated.[49–52] One factor that may mitigate against the theoretic value of IOCM versus LOCM is that IOCM is a dimer and has a higher viscosity than LOCM. Viscosities for HOCM, LOCM, and IOCM are 0.00275, 0.00525, and 0.0114 pascal-second (Pa·s) at 37° C, respectively.[28] According to Poiseuille's law, an increase in viscosity negatively affects flow. Following intravenous injection, contrast medium becomes diluted in the bloodstream, the viscosity and osmolality are reduced, and therefore nonkidney organs are exposed to low concentrations of contrast medium. In the kidney, however, because of the increase in medullary osmolality, the concentration of contrast medium increases in the peritubular capillary and, following filtration, the concentration of contrast rises in the tubule lumen. The consequence is that (1) the distal tubules are exposed to increasing concentration and viscosity of contrast medium, and (2) the tubule flow rate decreases, leading to prolonged exposure to contrast, potentially enhancing direct tubule nephrotoxicity.[28,53] Furthermore, when infused in animals, medullary Po_2 is lower in IOCM when compared with LOCM.[34]

Aminoglycoside Nephrotoxicity. The nephrotoxicity of aminoglycosides has best been characterized for gentamicin, a polar drug excreted by glomerular filtration. It is thought that cationic amino groups (NH_3^+) on the drug bind to anionic phospholipid residues on the brush border of proximal tubular cells, and the drug is then internalized by endocytosis. Although the precise cellular mechanisms responsible for renal accumulation of aminoglycosides have not been fully elucidated, binding to the endocytic complex formed by megalin and cubilin at the apical surface of proximal tubular cells appears important.[54,55] The complete elimination of aminoglycoside uptake in mice deficient in megalin has suggested that this is the major pathway responsible for renal aminoglycoside accumulation.[55] Chloride transporters, including cystic fibrosis transmembrane conductance regulator (CFTR) and the ClC-5 have been implicated because mice lacking functional CFTR ($Cftr^{\Delta F/\Delta F}$) or deficient in the Cl^-/H^+ exchanger ($Clcn5^{Y/-}$) have decreased kidney accumulation of gentamicin.[56] A three-dimensional model has described the complex between megalin and gentamicin. Gentamicin binds to megalin with low affinity and exploits the common ligand-binding motif using the indole side chain of amino acids Trp-1126 and the negatively charged residues of Asp-1129, Asp-1131, and Asp-1133.[57] Once endocytosed, aminoglycosides inhibit endosomal fusion. They are also directly trafficked to the Golgi apparatus and, through retrograde movement, to the endoplasmic reticulum (ER). From the ER, gentamicin moves into the cytosol in a size- and charge-dependent manner.[58] Once in the cytosol, either from the ER[58] or via lysosomal rupture, aminoglycosides distribute to various intracellular organelles and mediate organelle-specific toxicity, such as mitochondrial dysfunction.[58,59] Gentamicin acts on the mitochondria, activating the intrinsic pathway of apoptosis, disrupting ATP production,[60,61] and producing hydroxyl radicals and superoxide anions.[62,63] Also, delivery to the ER via retrograde transport from the Golgi apparatus allows for the binding of aminoglycosides to the 16S rRNA subunit,[64] resulting in a reduction of protein synthesis[65,66] and altering posttranslational protein folding.[67] The number of cationic groups on the molecules determines the facility with which these drugs are transported across the cell membrane and is an important determinant of toxicity.[68,69] Neomycin is associated with the most nephrotoxicity, gentamicin, tobramycin, and amikacin are intermediate, and streptomycin is the least nephrotoxic. Furthermore, blocking megalin with cilastatin is known to reduce drug-induced nephrotoxicity.[70] Risk factors for aminoglycoside nephrotoxicity include the use of high or repeated doses or prolonged therapy, CKD, volume depletion, diabetes, advanced age, and the coexistence of renal ischemia or use of other nephrotoxins.[71–73]

Vancomycin Nephrotoxicity. Vancomycin was discovered, developed, and approved by the US Food and Drug Administration (FDA) in 1958[74,75] and was found to be active against most gram-positive organisms, including penicillin-resistant staphylococci.[75] Initial formulations of the drug were dubbed "Mississippi mud" due to the brown color presumably due to impurities, which were thought to be responsible for

nephrotoxicity and ototoxicity.[75–77] Reformulation resulted in a drug that had improved purity and was called *vancomycin* (from the word vanquish).

The incidence of vancomycin nephrotoxicity is variable, ranging from as low as 0% to over 40%, and those with vancomycin-associated nephrotoxicity were more likely to have higher trough levels and prolonged duration of treatment.[78] Durations of 7 to more than 15 days of treatment are associated with nephrotoxicity.[79–81] With modern formulations of vancomycin, the variability is due to concomitant drug use, severity of illness, and variability in the definition of AKI. Daily total dose of vancomycin of more than 4 g has been shown to be a risk factor for nephrotoxicity.[82] Vancomycin-associated nephrotoxicity is thought to be more common when combined with antipseudomonal beta-lactams. In a retrospective matched cohort study of patients receiving vancomycin and cefepime (588 patients) or vancomycin and piperacillin-tazobactam (3605 patients), the unadjusted incidence of AKI was 12.6% versus 21.4%, respectively ($P < .0001$). Vancomycin and piperacillin-tazobactam was associated with a more than twofold increase in the risk of AKI, after matching for severity of illness.[80]

Luque and associates[83] have described a patient who received vancomycin without coadministration of an aminoglycoside. The biopsy showed obstructive tubular casts composed of noncrystal nanospheric vancomycin aggregates associated with uromodulin. In eight additional patients with AKI associated with high vancomycin levels, vancomycin-associated casts were found. These findings, which were reproduced in mice given vancomycin, demonstrate a link between vancomycin and AKI.

Cisplatin Nephrotoxicity. Treatment with cisplatin (cisplatinum), a platinum-based chemotherapeutic agent, is commonly associated with nephrotoxicity. The pathophysiologic mechanism of cisplatin-induced tubular damage is complex and involves a number of interconnected factors, such as accumulation of cisplatin mediated by membrane transport, conversion into nephrotoxins, DNA damage, mitochondrial dysfunction, oxidative stress, inflammatory response, activation of signal transducers and intracellular messengers, and activation of apoptotic pathways. Movement of cisplatin through the renal tubular cells occurs in a basolateral to apical direction. Two primary transporters are involved in transporting cisplatin into the tubular cells, the copper transport protein 1 (Ctr1), expressed on proximal and distal tubules, and the organic cation transporter 2 (OCT2), expressed on the basolateral side of the proximal convoluted tubule.[84] Multidrug and toxin extrusion 1 (MATE1/SLC47A1; MATE1), expressed on the apical membrane of the proximal tubule, is responsible for the efflux of cisplatin.[84,85] When cisplatin was administered to *Mate1*$^{-/-}$ mice, blood urea nitrogen (BUN) and creatinine levels were higher than in *Mate1*$^{+/+}$ mice. Furthermore cisplatin levels were higher in plasma and in kidneys of compared with *Mate1*$^{+/+}$ mice, suggesting that MATE1 mediates the efflux of cisplatin and could contribute to cisplatin nephrotoxicity.

The S3 segment of the proximal tubule in the corticomedullary region is the most common site of cisplatin nephrotoxicity in rats. More distal sites may be affected in humans, but glomeruli remain unaffected. A recent study in mice has examined the effects of low but frequent doses of cisplatin given once a week for 4 weeks. Mice who received multiple doses of cisplatin had increased levels of fibrotic markers in kidney tissue, including fibronectin, transforming growth factor-β, and α-smooth muscle actin, as well as interstitial fibrosis.[86] These studies in mice support observations in humans showing that adult patients with cancer who received multiple doses of experience small but permanent declines in the estimated GFR (eGFR).[87,88]

Acute Phosphate Nephropathy. AKI has been described as a complication following the administration of oral sodium phosphate solution as a bowel cathartic in preparation for colonoscopy and bowel surgery.[89–91] Although the mechanism linking oral sodium phosphate administration with AKI remains incompletely understood, the pathogenesis likely relates to a transient and significant rise in the serum phosphate concentration that occurs simultaneously with intravascular volume depletion.

When the urine is oversaturated and buffering factors such as pH, citrate, and pyrophosphate are overwhelmed, renal phosphorus excretion becomes compromised. This may lead to the intratubular precipitation of calcium phosphate salts when the solubility coefficient is exceeded and to obstruction of the tubular lumen, leading to direct tubular damage. ROS generated by the binding of calcium phosphate crystals further promotes tubular damage. Risk factors for acute phosphate nephropathy include preexisting volume depletion, the use of ACE inhibitors and angiotensin receptor blockers (ARBs), NSAIDs, CKD, older age, female gender, and higher dosage of oral sodium phosphate.[90,91] Patients who develop acute phosphate nephropathy typically present with elevated serum creatinine concentrations days to months following the administration of oral sodium phosphate solution and can experience progression to CKD and end-stage kidney disease. As in other conditions associated with hypercalcemia, hyperphosphatemia, or hyperphosphaturia, calcium phosphate precipitation in renal tubules is seen on renal biopsy as bluish-purple crystals that are nonpolarizable.[92]

Tubular Disease—Endogenous Nephrotoxins

Myoglobin and Hemoglobin. Myoglobin and hemoglobin are the endogenous toxins most commonly associated with ATN. Myoglobin, a 17.8-kDa heme protein released during muscle injury, is freely filtered and causes red-brown urine, with a dipstick result positive for heme in the absence of RBCs in the urine. Intravascular hemolysis results in circulating free hemoglobin, which, when excessive, is filtered, resulting in hemoglobinuria, hemoglobin cast formation, and heme uptake by proximal tubular cells. The uptake in the proximal tubule may be mediated via endocytosis by megalin and cubilin. Megalin knockout mice have reduced accumulation of injected myoglobin in tubule cells and reduced nephrotoxicity.[93] The heme center of myoglobin may directly induce lipid peroxidation and, in addition, the liberation of free ferrous iron, depending on the redox potential, can promote hydroxyl radical formation by the Haber-Weiss (Fenton) reaction, resulting in the oxidation of lipids, proteins, and nucleic acids.[94,95] Iron is an intermediate accelerator in the generation of free radicals. Studies have suggested that there is increased formation of H_2O_2 in rat kidney models of myohemoglobinuria.[96] The subsequent hydroxyl

(OH^-) radical plays a vital role in oxidative stress–induced AKI through mechanisms discussed in detail later in this chapter. In response, heme protein induces heme-degradative enzyme, heme oxygenase, and increased synthesis of ferritin. Ferritin, a major factor in sequestering free iron,[97] is made up of two types of 24 subunits, heavy chain and light chain. It is the ferritin heavy chain (FtH) that has ferroxidase activity necessary for iron incorporation and to limit toxicity. Zarjou and coworkers have demonstrated that proximal tubule-specific, *FtH*-knockout mice ($FtH^{PT-/-}$ mice) have significant mortality in myoglobin-induced AKI, indicating the protective role of proximal tubule FtH in AKI.[98] Various iron chelators such as deferoxamine and other scavengers of ROS such as glutathione provide protection against myohemoglobinuric AKI.[99] Similarly, endothelin antagonists also prevent hypofiltration and proteinuria in rats that have undergone glycerol-induced rhabdomyolysis.[100] In addition, NO supplementation may be beneficial by preventing the heme-induced renal vasoconstriction because heme proteins scavenge NO.[101,102] Finally, precipitation of myoglobin with Tamm-Horsfall protein and shed proximal tubular cells lead to cast formation and tubular obstruction, which is enhanced in acidic urine.[103] In human studies, volume expansion and perhaps alkalization of urine to limit cast formation are the preventive measures generally used because none of the experimental agents used in animal studies has been convincingly beneficial. This emphasizes the multifactorial nature of these conditions. It is unlikely that a single agent will be beneficial in this setting.[104]

Immunoglobulin Light Chains. *Direct tubule toxicity.* Excessive immunoglobulin light chains, produced in diseases such as multiple myeloma, are filtered, absorbed, and then catabolized in proximal tubule cells and can induce proximal tubulopathy.[105] The concentration of light chains leaving the proximal portion of the nephron depends on both the concentration of light chains in the glomerular filtrate and the capacity of the proximal tubule to reabsorb and catabolize them. Certain light chains can be directly toxic to the proximal tubules themselves.[106] In the proximal tubules, free light chains (FLCs) are reabsorbed by binding to the proximal tubule heterodimeric complex consisting of megalin and cubilin.[107–109] Accumulation of light chains in the endosomes and lysosomes of the proximal tubule leads to cellular desquamation and fragmentation, vacuolization, and focal loss of brush border.[110] Mechanisms for tubule toxicity may include blocking of transport of glucose, amino acids, or phosphate.[105] FLCs generate hydrogen peroxide,[111] which leads to the production of chemokines and cytokines,[111–114] with nuclear translocation of nuclear factor-kappa B (NF-κB), suggesting that light chain endocytosis leads to the production of inflammatory cytokines through activation of NF-κB.[115] Monoclonal FLC also promotes apoptosis through apoptosis signal-regulating kinase (ASK1), also called mitogen-activated protein kinase kinase kinase 5 (MAP3K5).[114] Subsequent inflammation leads to tubulointerstitial fibrosis.[116]

Cast nephropathy. Once the capacity for proximal tubule uptake is overwhelmed, a light chain load is presented to the distal tubule, where, on reaching a critical concentration, the light chains aggregate and coprecipitate with Tamm-Horsfall protein (THP) and form characteristic light chain casts.[117] FLCs bind to specific sites on Tamm-Horsfall glycoproteins through the CDR3 domain of FLC, leading to their coprecipitation in the lumen of the distal nephron and tubule flow.[118] There are critical determinants of the binding site between CDR3 and FLCs that lead to the development of a cyclized competitive peptide. This peptide inhibited binding of FLCs to THP and was effective in inhibiting intraluminal cast formation and AKI.[118] Some studies have shown that light chains, in the amount seen in plasma cell dyscrasia patients, are capable of catalyzing the formation of hydrogen peroxide in cultured HK-2 cells. Hydrogen peroxide stimulates the production of monocyte chemoattractant protein-1 (MCP-1), a key chemokine involved in monocyte or macrophage recruitment to proximal tubular cells.[111]

Any process that reduces the GFR, such as volume depletion, hypercalcemia, or NSAIDs, will accelerate and aggravate light chain cast formation. It has been proposed that acutely reducing the presented light chain load by plasmapheresis or dialysis using high-cutoff membranes might be beneficial in limiting cast formation and reducing the extent of AKI in certain select patients, allowing for the initiation of chemotherapy to decrease bone marrow– dependent light chain formation.[119,120]

Uric Acid. Tumor cell necrosis following chemotherapy can release large amounts of intracellular contents such as uric acid, phosphate, and xanthine into the circulation, potentially leading to AKI. Acute uric acid nephropathy with intratubular crystallization leading to obstruction and interstitial nephritis is not seen as commonly as it was in the past, mainly due to the prophylactic use of allopurinol or rasburicase before chemotherapy to lower the serum uric acid concentration acutely.

POSTRENAL ACUTE KIDNEY INJURY

Postrenal azotemia occurs from obstruction of the ureters, bladder outlet, or urethra. AKI from ureteric obstruction requires that the blockage occur bilaterally at any level of the ureters or unilaterally in a patient with a solitary functioning kidney or CKD. Ureteric obstruction can be intraluminal or external. Bilateral ureteric calculi, blood clots, and sloughed renal papillae can obstruct the lumen, whereas external compression from a tumor or hemorrhage can block the ureters as well. Fibrosis of the ureters intrinsically or from the retroperitoneum can narrow the lumen to the point of complete luminal obstruction. The most common cause for postrenal azotemia is structural or functional obstruction of the bladder neck. Prostatic conditions, therapy with anticholinergic agents, and a neurogenic bladder can all cause postrenal AKI. Relief of the obstruction usually causes prompt return of the GFR if the duration of obstruction has not been excessive. The rate and magnitude of functional recovery are dependent on the extent and duration of the obstruction.[121]

AKI resulting from obstruction usually accounts for fewer than 5% of cases, although in certain settings (e.g., transplantation), it can be as high as 6% to 10%. Clinically, patients can present with pain and oliguria, although these are neither specific nor sensitive. Because of the availability of retroperitoneal imagining using ultrasonography or computed tomography (CT), the diagnosis is usually straightforward, although, on occasion, a volume-depleted patient or a patient

with severe reduction in the GFR may not show hydronephrosis on radiologic assessment. Because the GFR is typically not affected early in the course of obstructive AKI, volume repletion can increase the sensitivity of diagnosis by increasing the GFR and urine production into the ureter, leading to dilation of the ureter proximal to the obstruction, enhancing ultrasonographic visualization. Early diagnosis and prompt relief of obstruction remain key goals in preventing long-term parenchymal damage because the shorter the period of obstruction, the better the chances for recovery and favorable long-term outcomes.

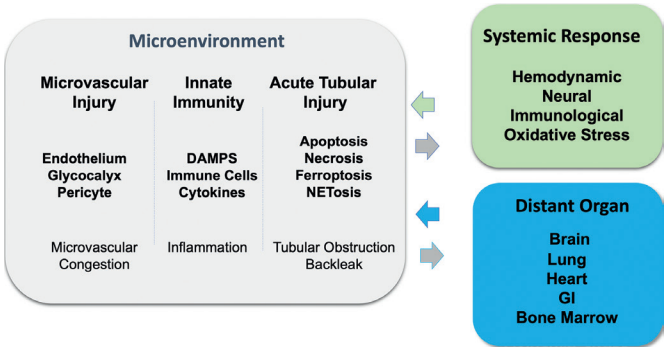

Fig. 28.1 Overview of pathophysiology of acute kidney injury (AKI). AKI is a summation of temporally activated systems that together result in loss of the glomerular filtration rate (GFR). In the kidney, mechanisms pertaining to microvascular compartment, innate immunity, and acute tubular necrosis result in temporary, partial, or permanent loss of the GFR. Systemic responses may influence the extent of injury. Hemodynamic alterations (decrease in cardiac output, low blood pressure, and vasoconstriction) may initiate AKI or exacerbate intrinsic microenvironmental mechanisms of AKI. Systemic immunologic mechanisms of proinflammatory or antiinflammatory conditions may affect AKI, and neural mechanisms may attenuate AKI (see text for details).

PATHOPHYSIOLOGY OF ACUTE KIDNEY INJURY

OVERVIEW OF THE PATHOPHYSIOLOGY OF ACUTE KIDNEY INJURY

AKI is a summation of temporally activated systems that together result in inflammation, activation of cell death pathways, tubular obstruction, backleak, altered glomerular hemodynamics and loss of the GFR. Within the kidney, mechanisms pertaining to microvascular compartments, innate immunity, and ATN result in temporary, partial, or permanent loss of the GFR. Furthermore, it is becoming widely accepted that AKI is a systemic process that affects a number of organs, leading to the high morbidity and mortality seen in patients with AKI. Systemic responses to AKI may influence the extent of injury. Hemodynamic alterations (e.g., decrease in cardiac output, low blood pressure, vasoconstriction) may initiate AKI or exacerbate intrinsic microenvironmental mechanisms of AKI. Systemic immunologic mechanisms of proinflammatory or antiinflammatory conditions may affect AKI, and neural mechanisms may attenuate AKI. Thus, the complexity of the pathogenesis of AKI requires careful understanding of its molecular mechanisms through defining important targets in humans and testing in relevant models of AKI (Fig. 28.1).[122–127]

EXPERIMENTAL MODELS

The goal of preclinical AKI research is to translate basic scientific knowledge of AKI to clinical practice. Despite extensive research in AKI focusing on standardized definitions of AKI (from *R*isk, *I*njury, *F*ailure, *L*oss of kidney function, and *E*nd-stage kidney disease (RIFLE), *A*cute *K*idney *I*njury *N*etwork (AKIN), and Kidney Disease: Improving Global Outcomes [KDIGO]), biomarkers of AKI, novel drug targets, and improved clinical trial design, our knowledge remains incomplete, and effective therapies are lacking.[122,123] To advance the field, it is important that we identify relevant targets through the analysis of human tissue biopsy and necropsy specimens, develop relevant disease models of AKI, include proper pharmacokinetic, pharmacodynamic, and dose response studies and, finally, improve preclinical and human clinical trial design.[123,125,126,128] The National Institutes of Health (NIH) and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) have initiated a bold project, the Kidney Precision Medicine Project (KPMP), whose objectives are to obtain human kidney biopsies ethically from participants with AKI and CKD with a goal to identify critical cells, pathways, and targets for therapy. The following section will focus on current animal models.

IN VIVO MODELS OF ACUTE KIDNEY INJURY

Current investigations into the pathophysiology of AKI include a combination of animal and cell culture models of AKI designed to understand the pathophysiology of AKI better and investigate novel therapeutic agents. However, there remains a need to develop in vivo experimental models of AKI that more closely resemble clinical AKI for the development of effective therapies.[123,126,129,130] Some of the important principles in studying the pathophysiology of AKI in various models include outcome measures at multiple time points and the ability to control physiologic functions known to affect kidney function (e.g., temperature, blood pressure, anesthesia, fluid status). Current models of AKI (e.g., warm ischemia-reperfusion using a pedicle clamp) test fundamental proof of principle concepts that identify potential molecular targets. A simplified model limits confounding variables but can clearly identify potential therapeutic targets. However, additional models of AKI are necessary that should reflect human disease (e.g., aged animals, impaired kidney function, multiorgan failure, preexisting vascular changes, multiple renal insults) that often coexist in human AKI. We will briefly describe the pros and cons of using presently characterized experimental models (Table 28.1).[131–146]

The warm ischemia-reperfusion renal pedicle clamp model is one of the most widely used experimental models in rats and mice because of its simplicity and reproducibility. However, the inflammatory response differs greatly between mice and rats. It is important to realize that there is considerable variability between mice and rats, mouse strains (C57BL/6 vs. BALB/c), and the same strain from different vendors.[147] Although mice are the primary species used experimentally for examining the immune response to AKI, there are significant differences when compared with the human immune

Table 28.1. Acute Kidney Injury–Induced Distant Organ Injury

Organ	Mechanism	Species	Reference
Lung	CXCL1	Mouse	131
	TNF-α	Mouse	132
	IL-10	Mouse	133
	Neutrophil elastase	Mouse	134
	Lung permeability	Mouse	135
	Pulmonary edema, inflammatory cytokines	Rat	136
	Oxidant stress	Rat	137
	Systemic cytokines and HMGB1	Human (ex vivo)	138
Heart	TNF-α	Rat	139
	Mitochondrial fission protein	Mouse	140
Brain	RAAS	Mouse	141
Liver	Administration of glutathione	Rat	142
	Hepatic oxidant stress	Mouse	143, 144
Intestine	Hepatic oxidant stress	Mouse	145

AKI, Acute kidney injury; *CXCL1,* chemokine (C-X-C motif) ligand 1; *HMGB1,* high-mobility group box protein B1; *IL,* interleukin; *RAAS,* renin-angiotensin-aldosterone system; *TNF-α,* tumor necrosis factor α.

Modified from Lee SA, Cozzi M, Bush EL, Rabb H. Distant organ dysfunction in acute kidney injury: a review. *Am J Kidney Dis.* 2018;72(6):846–856.

system.[148] Additionally, there are structural differences between rodents and humans, although pig kidneys are most similar to humans.[130]

These differences need to be taken into consideration when using mice in preclinical models to mimic human AKI. Furthermore, tubular injury and repair and medullary congestion are difficult to compare with human ischemic ATN. In human AKI, pure ischemia alone is seen in the minority of cases, and there is rarely complete cessation of blood flow to the kidneys. The parenteral delivery of prophylactic therapeutic agents is impossible in complete occlusion models. Because oxygen and metabolic substrates are unable to reach the kidney, the generation of ROS and peroxynitrite species, considered to be important mediators of injury, might have a different or delayed role as compared with low-oxygen states in hypoperfusion models. Total blood flow cessation also prevents the degradative products of the ischemic kidney from being washed out. Other factors playing a role in the pathophysiology of AKI, such as inflammatory mediators released from the spleen, ischemic gut, endothelium, and vascular smooth muscle cells, need to be taken into consideration in any experimental model. Bowel proteins released into the circulation can act as inflammatory mediators and increase the susceptibility to AKI.[149] Others have shown that short-chain fatty acids derived from gut bacteria prevent AKI[150] or germ-free conditions render mice more susceptible to AKI.[151,152] The S3 segment of the proximal tubule is characterized by severe necrosis in clamp models, a finding seen rarely in human AKI. Human biopsies, however, rarely sample the outer medulla, where most of the injury is thought to occur. In contrast to animal models, human AKI histologic biopsy data are lacking at early time points from the onset of insult.[129] Thus, the NIH/NIDDK KPMP will be vital in this regard.

The cold ischemia–warm reperfusion model resembles human kidney transplantation but is inadequately studied and experimentally difficult to perform. In the isolated perfused kidney model, the kidney is perfused ex vivo using perfusates with and without erythrocytes, and the model uses ischemic (stopping perfusate) or hypoxic (reduced oxygen tension of erythrocytes) to induce functional impairment. The morphologic patterns are different in erythrocyte-free and erythrocyte-rich perfusates. The latter system is more comparable with what is observed histologically in animal models. Additionally, limitations include exclusion of various inflammatory mediators, neuroendocrine hemodynamic regulation, and systemic cytokine and growth factor interactions known to be present and that play a pathophysiologic role in animal models and likely in human ischemia.

Cardiac arrest commonly leads to AKI. Burne-Taney and colleagues have described a whole-body ischemia-reperfusion injury (IRI) model induced by 10 minutes of cardiac arrest, followed by cardiac compression resuscitation, ventilation, epinephrine, and fluids that lead to a significant rise in serum creatinine level and renal tubular injury at 24 hours.[153] One of the unique advantages of this model is the crosstalk among vital organs such as the brain, heart, and lung and renal hemodynamics.[139] A hypoperfusion model of AKI using partial aortic clamping, first described by Zager,[154] may be more representative of human AKI, reflecting a state of reduced blood flow to the kidney, with systolic blood pressure of approximately 20 mm Hg, resulting in reproducible AKI.[154]

Toxic models of kidney failure use various known toxins, such as radiocontrast media, gentamicin, cisplatin, glycerol, and pigments, including myoglobin, folic acid, and hemoglobin. Septic models to study AKI include cecal ligation and puncture (CLP), endotoxin infusion, and bacterial infusion into the peritoneal cavity. The endotoxin model, which is simple, inexpensive, and suitable for studying new pharmacologic agents, has certain drawbacks as well. There is variability among sources of lipopolysaccharide (LPS) endotoxin, the rates and methods of administration vary, and it is usually of short duration due to the high mortality associated with the doses required to induce AKI. It also

tends to be a vasoconstrictive model and does not recapitulate the early hemodynamics or inflammation of human sepsis.[155] Wichterman and colleagues were the first to describe a sepsis model in the early 1980s using the CLP laboratory model.[156] In the CLP model, there is considerable similarity to sepsis in humans, with acute lung injury, metabolic derangement, and systemic vasodilation, accompanied initially by increased cardiac output. However, there is some variability depending on the mode and size of cecal perforation. Doi and coworkers have developed a sepsis model that considers the following: (1) animals should receive the same supportive therapy that is standard for intensive care unit (ICU) patients (fluid resuscitation and antibiotics); and (2) age, chronic comorbid conditions, and genetic heterogeneity vary.[157] Another example is a hyperdynamic form of sepsis established in Merino sheep. These studies have provided important new information that cannot be derived from rodent models of sepsis.[158] Complex animal models of human sepsis that introduce these disease-modifying factors are likely more relevant and may be more pharmacologically relevant than simple animal models.[157]

This description is intended to remind the reader of the potential pitfalls in each model when evaluating experimental studies or therapeutic interventions using these models. The lack of ability to demonstrate the effectiveness of an agent in humans shown to be efficacious in animal models does not necessarily reflect a flaw with the model or the agent in question. Most often, the agent is administered late in the course of the human disease; patient heterogeneity and the difficulty in stratifying patients by severity of injury makes it even more difficult to establish efficacy.[123,159] Further studies have led to the development of zebrafish, three-dimensional (3D) microfluidic, and human kidney organoids models that are improving our understanding of AKI or facilitating pharmacologic studies for the treatment of AKI.

Zebrafish

Given the capacity of zebrafish adult nephrons to undergo robust epithelial regeneration and to form nephrons de novo, investigations using zebrafish models of AKI have allowed a better understanding of the cellular mechanisms associated with kidney regeneration after AKI. Using chemical genetics, investigators have discovered that histone deacetylase inhibitors (HDACi) are capable of ameliorating gentamicin-induced AKI in the zebrafish embryo with an expansion of renal progenitors that express the genes *Lhx1a*, *Pax2a*, and *Pax8*.[160] Future studies using zebrafish transgenic lines in which injury can be induced in tubular cells via the nitroreductase-metronidazole system, are likely to help characterize how individual populations of cells in the nephron respond to kidney damage.[161]

Three-Dimensional Microfluidic Models and Organoids

Side effects, and especially drug-induced nephrotoxicity, can often be an important limiting factor in the development of new pharmacotherapy. In addition to each of the current animal models of AKI having limitations in fully recapitulating human AKI, the lack of ability to predict drug-induced AKI has led to failed drug trials. Early nephrotoxicity screening studies included two-dimensional standard well plates with semipermeable filter cups. There were a number of limitations to two-dimensional standard well plates, including the following: (1) use of cell lines of nonhuman origin (MDCK; LLC-PK 1); (2) use of cell lines with limited proximal tubule characteristics (human kidney 2 [HK 2]); (3) cell lines that have features of epithelial to mesenchymal transition (HK 2); and (4) static conditions. By contrast, proximal tubule cells are subject to continuous tubule fluid flow and fluid shear stress that modulate cellular signal transduction through mechanosensing receptors, organization of the cytoskeleton, actin filaments, and adherens junctions.[162–166] More physiologically relevant models such as three-dimensional microfluidic models have been developed to bridge the step from standard two-dimensional systems to animal models or as a total replacement for costly, inefficient animal models. These three-dimensional microfluidic models, such as parallel flow-plate models and early three-dimensional perfusion models, include chip technology with cell monolayers or tubular structures that are sandwiched between two microfluidic channels. Recently, Qu and coworkers developed a multilayer microfluidic device to simulate glomerulus, Bowman's capsule, proximal tubular lumen, and peritubular endothelial cells to investigate the pathophysiology of drug-induced AKI. The authors were able to demonstrate time- and dose-dependent induction of cell death by cisplatin and doxorubicin. The use of this biomimetic device has yielded useful information about drug-induced AKI at the preclinical stage.[167]

Human kidney organoids are three-dimensional clusters of cells that are functionally and genetically similar to kidney. Human-induced pluripotent stems cells (hiPSCs) are ideally suited for human kidney organoids because of their unlimited self-renewal and ability to generate cells of all three embryonic germ layers. Major challenges to the generation of functional bioengineered kidneys are the incorporation of adequate vascularization to kidney organoids and the establishment of an effective drainage system for the removal of blood filtrate after passage and processing through the tubule system[168] Protocols that add combinations of growth factors to mimic in vivo conditions and growing cells in three-dimensional cultures have generated nephron progenitor cells and kidney organoids from human embryonic stem cells and hiPSCs.[169–172]

ACUTE TUBULAR NECROSIS

EPITHELIAL CELL INJURY

Although all segments of the nephron may undergo injury during an ischemic insult, the major and most commonly injured epithelial cell involved in AKI related to ischemia, sepsis, and/or nephrotoxins is the PTC. Of the three segments (S1–S3), the S3 segment of the proximal tubule in the outer stripe of the medulla is the cell most susceptible to ischemic injury for several reasons.[173] First, it has limited capacity to undergo anaerobic glycolysis due to its dependence on fatty acid oxidation as the major source of energy. Second, due to its unique primarily venous capillary regional blood flow, there is marked hypoperfusion and congestion in this medullary region after injury that persists, even though cortical blood flow may have returned to near-normal levels after ischemic injury. Endothelial cell injury and dysfunction are primarily responsible for this phenomenon, often referred to as the "extension phase" of AKI.[159] The other major epithelial cells of the nephron involved are those of the medullary

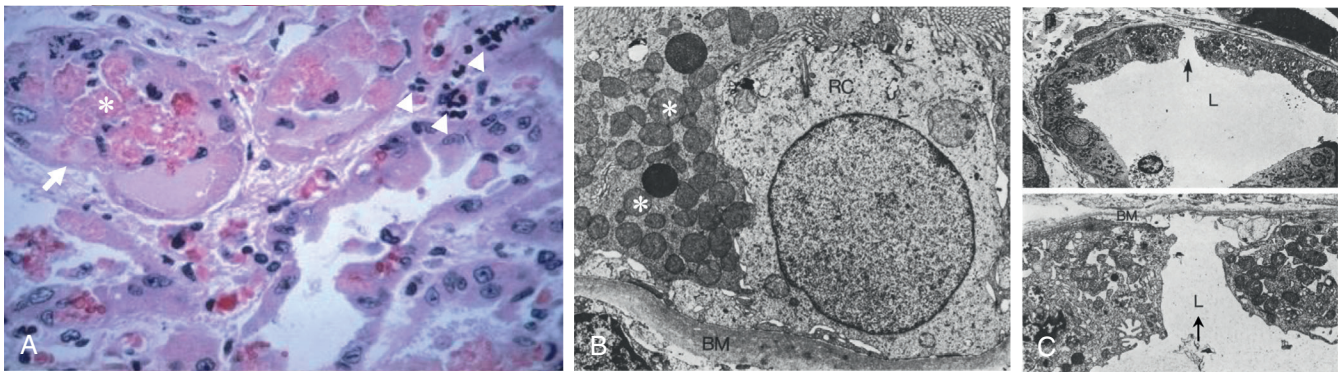

Fig. 28.2 Morphology of human acute tubular necrosis. (A) Human biopsy specimens reveal significant proximal tubular cell damage, with intraluminal accumulation of apical membrane fragments and detached cells *(*),* thinning of proximal tubular cells to maintain monolayer tubule integrity *(arrow),* and dividing cells and accumulation of white cells within the microvascular space in the peritubular area *(arrowheads).* (B) Electron micrograph of a regenerating epithelial cell. Shown are small fragmented mitochondria *(*)*. (C) Electron micrograph of renal epithelial cell showing nonreplacement site *(black arrow)* that morphologically supports the concept of backleak in the pathophysiology of AKI. (A, Courtesy M. Venkachatalam; B and C from Olsen TS, Olsen HS, Hansen HE. Tubular ultrastructure in acute renal failure in man: epithelial necrosis and regeneration. *Virchows Arch A Pathol Anat Histopathol.* 1985;406(1):75–89.)

thick ascending limb located more distally. Cells of the S1 and S2 segments are usually involved in toxic nephropathy due to their high rates of endocytosis, leading to increased cellular uptake of the toxin. PTCs and thick ascending limb of Henle (TAL) cells have been shown to be involved as sensors, effectors, and injury recipients of AKI stimuli.

Proximal tubular cell injury and dysfunction during ischemia or sepsis lead to a profound drop in the GFR through afferent arteriolar vasoconstriction, mediated by tubular glomerular feedback and proximal tubular obstruction. This phenomenon, along with tubular backleak, leads to a fall in the effective GFR[174,175] (Figs. 28.1 and 28.2).

Morphologic Changes

The classic histologic hallmark of ATN was described in a landmark study by Oliver and coworkers, in which individual nephrons from autopsy specimens of patients dying of acute renal failure (ARF) were microdissected. Portions of glomerular ultrafiltrate had become sequestered in tubules that were obstructed (necrotic PT cells), which suggested that filtrate leaked back through damaged tubular walls and entered the interstitium, which caused it to become edematous.[176] Early on, there is the loss of the apical brush border of the PTCs. Microvilli disruption and detachment from the apical cell surface forming membrane-bound blebs occurs early, with release into the tubular lumen. Patchy detachment and subsequent loss of tubular cells exposing areas of denuded tubular basement and focal areas of proximal tubular dilation, along with the presence of distal tubular casts, are also major pathologic findings in ATN.[177] The sloughed tubular cells, brush border vesicle remnants, and cellular debris in combination with Tamm-Horsfall glycoprotein form the classic muddy brown granular casts. These distal casts have the potential to obstruct the tubular lumen. Frank necrosis itself is inconspicuous and is restricted to the highly susceptible outer medullary regions. Alternatively, features of apoptosis are more commonly seen in proximal and distal tubular cells. Glomerular epithelial cell injury in ischemic, septic, or nephrotoxic injury is not typically seen, although some studies have shown thickening and coarsening of foot processes; Wagner and associates have shown podocyte-specific molecular and cellular changes.[189] The future morphologic course of the tubular cell alterations varies according to the type and extent of injury, as discussed in the next section (see Fig. 28.2).

Cytoskeletal and Intracellular Structural Changes

The cytoskeleton in eukaryotic cells consists of intermediate filaments, microtubules, and actin filaments.[178] The microtubule cytoskeleton is composed of α-tubulin and β-tubulin heterodimers that serve to regulate the shape, motility, and division of tubular epithelial cells. A recent study has demonstrated that IRI to the kidney causes α-tubular deacetylation in microtubules, and inhibition of microtubule dynamics induced by changes in tubulin acetylation during the recovery phase retards tubular epithelial cell regeneration.[179] Epithelial cellular structure and function are mediated in part by the actin cytoskeleton, which plays an integral role in surface membrane structure and function, cell polarity, endocytosis, signal transduction, cell motility, movement of organelles, exocytosis, cell division, cell migration, barrier function of the junctional complexes, cell-matrix adhesion, and signal transduction.[180] Based on its role in this multitude of processes, any disruption of the actin cytoskeleton results in changes and/or disruption of the functions mentioned earlier. This is especially important for PTCs, where amplification of the apical membrane by microvilli is essential for normal cell function.

Ischemic insult results in cellular ATP depletion, which in turn leads to a rapid disruption of the apical actin and disruption and redistribution of the cytoskeleton F-actin core, resulting in the formation of membrane-bound extracellular vesicles or blebs.[181] These can be exfoliated into the tubular lumen or internalized with the capability of being recycled. The core mechanism of disruption is the depolymerization mediated by actin-binding protein known as actin depolymerizing factor (ADF) or cofilin.[182] This protein family is normally maintained in the inactive phosphorylated form, which cannot bind to actin. Ischemia results in ATP depletion, which has been shown to cause Rho GTPase inactivation.[183] This can

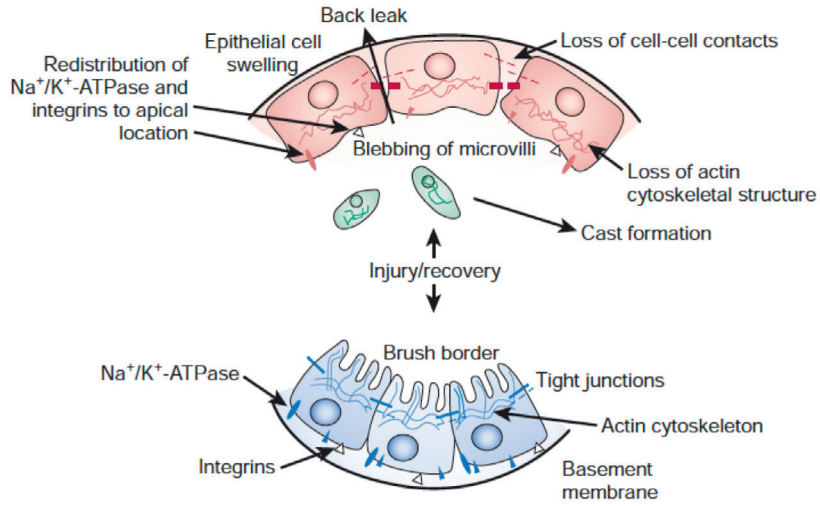

Fig. 28.3 Overview of sublethally injured tubular cells. Sodium-potassium adenosine triphosphatase (Na^+-K^+-ATPase) pumps are normally located at the basolateral membrane. In sublethal ischemia, the pumps redistribute to the apical membrane of the proximal tubule. On reperfusion, the pumps reverse back to their basolateral location. (From Sharfuddin A, Molitoris B. Epithelial cell injury. In Vincent JL, Hall JB, eds. *Encyclopedia of Intensive Care Medicine.* New York: Springer; 2012.)

lead to activation and relocalization of ADF (cofilin) to the apical membranes, where it can mediate different effects, including depolymerization, severing, capping, and nucleation of F-actin. This destroys the actin filament core structure of microvilli and results in surface membrane instability and blebbing[184,185] (Fig. 28.3).

A recent study has used human primary tubular epithelial cells in culture to examine the role of hypoxic injury on epithelial cell cytoskeletal organization. Given previous information that stabilization of hypoxia-inducible factor (HIF) simulates hypoxic injury during AKI, the authors found that HIF stabilization in human tubular epithelial cells reduces tubular cell migration and induces the rearrangement of actin filaments and cell adhesion molecules, including paxillin and focal adhesion kinase. These data support the role of HIF stabilization during epithelial migration, which underlies a potential mechanism of renal regeneration in response to AKI.[186] Similarly, tropomyosins physiologically bind to and stabilize the F-actin microfilament core in the terminal web by preventing access to ADF. After ischemia, there is dissociation of tropomyosins from the microfilament core, resulting in access of the microfilaments in the terminal web to the binding, severing, and depolymerizing actions of ADF/cofilin.[187,188]

Another important consequence of disruption of the actin cytoskeleton is the loss of tight junctions and adherens junctions. These junctional complexes actively participate in numerous functions, including paracellular transport, cell polarity, and cellular shape. The tight junctions, also known as zonula occludens (ZO), are composed of proteins such as occludin, claudin, ZO-1, and protein kinase C with numerous barrier functions, such as adhesion, permeability, and transport. The actin present in the terminal web is linked to ZO, and hence any disruption of the terminal web results in disruption of the tight junctions. Early ischemic injury results in opening of these tight junctions, leading to increased paracellular permeability and causing further backleak of the glomerular filtrate into the interstitium.[180] In the glomerulus, ischemia also induces a rapid loss of interaction between slit diaphragm junctional proteins NEPH1 and ZO-1,[189] leading to podocyte damage, effacement, and proteinuria.

The molecular mechanisms underlying these changes have been studied as well and show that ATP depletion that results in actin polymerization is followed by a reduction in cellular adhesion ability. Pretreatment with an actin stabilizer prevented ATP depletion–induced actin polymerization and reduction of cell adhesion, indicating that the cytoskeleton reorganization decreased the cellular adhesion ability. Furthermore, the ATP depletion markedly increased the levels of p38 mitogen-activated protein (MAP) kinase and heat shock protein 27 (hsp27) phosphorylation, with enhanced translocation of phosphorylated hsp27 from cytoskeleton to cytoplasm. The inhibition of p38 MAP kinase by specific inhibitor SB203580 blocked the ATP depletion to induce hsp27 phosphorylation and actin polymerization. These findings suggest that ischemia remodels F-actin, leading to desquamation of proximal tubular epithelial cells through p38 MAP kinase–hsp27 signaling.[190]

Actin cytoskeleton alterations and dysfunction during ischemia result in changes in cell polarity and function. Normally, sodium-potassium adenosine triphosphatase (Na^+-K^+-ATPase) pumps reside in the basolateral membrane of the tubular epithelial cell, but under conditions of ischemia, they redistribute to the apical membrane as early as within 10 minutes.[191] This process occurs due to the disruption of the pumps' attachment to the membrane via the spectrin/actin cytoskeleton. Postulated mediated mechanisms include hyperphosphorylation of the protein ankyrin, with consequent loss of the binding protein spectrin, and cleavage of spectrin by the activation of proteases such as calpain. This redistribution of the Na^+-K^+-ATPase pump results in bidirectional transport of sodium and water across the epithelial cell apical membrane, as well as the basolateral membrane. This results in transport of cellular Na back into the tubular lumen, one of the major mechanisms of the high FE_{Na} seen in patients with ischemic ATN,[192] and the inefficient use of cellular ATP. ATP is consumed but effective, vectorial Na transport is lost.

Cell Death Pathways

Necrosis and Regulated Cell Death Pathways. Epithelial cell necrosis is a passive nonenergy dependent process that develops secondary to severe ATP depletion from toxic or ischemic insult. It is not dependent on caspase activation but results from a rise in intracellular calcium and the activation of membrane phospholipases.[193,194] Hence, morphologically, necrotic cells do not exhibit the nuclear fragmentation or chromatin condensation seen in apoptosis, and they do not form apoptotic bodies. Functionally, severe ATP depletion results first in mitochondrial injury, with subsequent arrest of oxidative phosphorylation, causing further depletion of energy stores, and robust formation of ROS, which in turn mediate further cellular injury.

In some cases, defined regulated molecular pathways may lead to necrotic cell death, a process referred to as *necroptosis*.[195] Differentiation between necrosis and necroptosis requires that necroptosis-dependent cell death involves the receptor-interacting protein kinase 3 (RIPK3).[196] Regulated cell death pathways can be grouped into a caspase-controlled cell death system (apoptosis, necroptosis, and pyroptosis) or lipid per oxidation–autoxidation-controlled necrosis system (ferroptosis; Fig. 28.4).[197]

Apoptosis. The fate of an epithelial cell after an injury ultimately depends on the extent of the injury. Cells undergoing sublethal or less severe injury have the capacity for functional and structural recovery if the insult is interrupted. Cells that suffer a more severe (or lethal) injury undergo apoptosis or necrosis. Apoptosis is an energy-dependent, programmed cell death after injury that results in the condensation of nuclear and cytoplasmic material, forming apoptotic bodies. These apoptotic bodies, which are plasma-membrane bound, are rapidly phagocytosed by macrophages and neighboring viable epithelial cells. In necrosis, there is cellular and organelle swelling, with loss of plasma membrane integrity and release of cytoplasmic and nuclear material into the lumen or interstitium[198] (Fig. 28.5).

The caspase family of proteases is an important initiator as well as an effector of apoptosis.[199,200] Both the intrinsic (mitochondrial) and extrinsic (death receptor) apoptotic pathways are activated in human AKI. Specifically, activation of procaspase-9 primarily depends on intrinsic mitochondrial pathways regulated by the Bcl-2 family of proteins, whereas that of procaspase-8 results from extrinsic signaling via cell surface death receptors, such as Fas and their ligand FADD (Fas-associated protein with death domain). There is also considerable crosstalk between the intrinsic and extrinsic pathways. The other group of caspases—3, 6, and 7—are effector caspases, which are more abundant and catalytically robust, cleaving many cellular proteins and resulting in the classic apoptotic phenotype. Caspase activation in

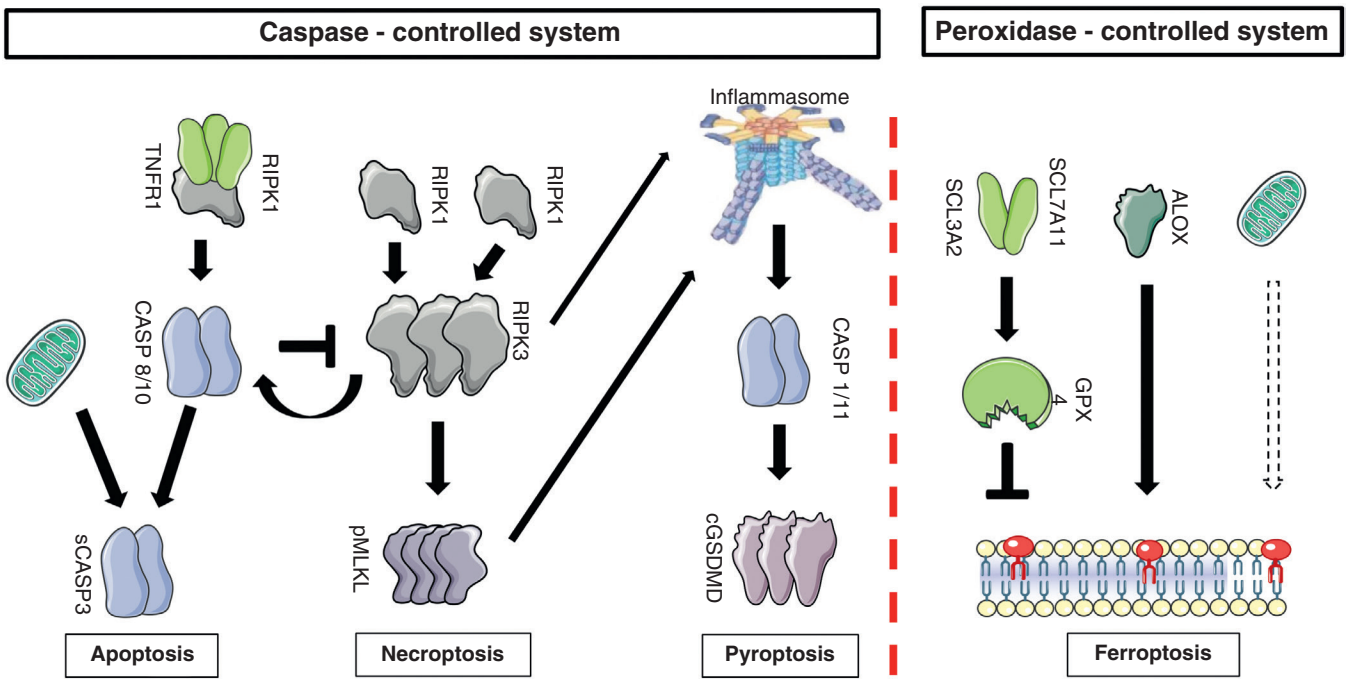

Fig. 28.4 Overview of the pathways of regulated cell death. In general, two systems may be best differentiated when regulated cell death is considered. The caspase-controlled system includes apoptosis, necroptosis, and pyroptosis and has been studied in great detail over the last few decades. In contrast, the peroxidation-controlled system of ferroptotic cell death functions entirely independently of the caspase-controlled network. Importantly, both systems contribute to human disease. Targeting clinically relevant cell death, therefore, should at least require a combination of therapies aimed at these two systems, which may exhibit some redundant functions. Similarly, in the caspase-controlled system, inhibition of either pathway may result in alternative pathways. With the idea of necroinflammation in mind, it may be helpful to shift a deadly signal from a highly immunogenic pathway (e.g., pyroptosis) toward a pathway with less immunogenic potency (e.g., apoptosis). (From Tonnus W, Gembardt F, Latk M, et al. The clinical relevance of necroinflammation—highlighting the importance of acute kidney injury and the adrenal glands. *Cell Death Differ.* 2019;26(1):68–82.)

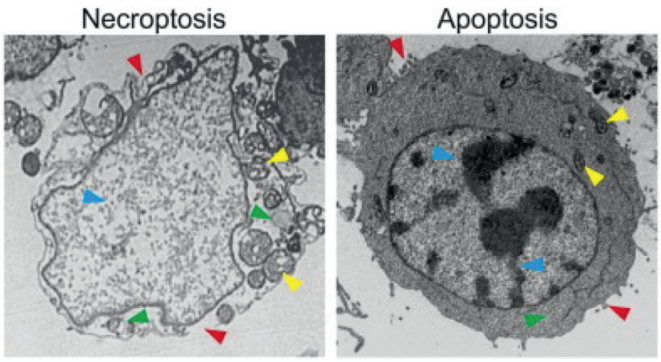

Fig. 28.5 Morphologic features of necroptosis and apoptosis. HT29 colon cancer cells treated with an anticancer drug for 48 hours were analyzed by transmission electron microscopy. The cell undergoing necroptosis shows plasma membrane rupture and permeabilization, compared with the intact plasma membrane, with blebbing in the apoptotic cell *(red arrowheads).* The necroptotic cell exhibits cytoplasm swelling and vacuolization, which are absent in the apoptotic cell *(green arrowheads).* The necroptotic cell has swelled mitochondria, in contrast to those in the apoptotic cells *(yellow arrowheads).* The necroptotic cell also lacks the condensed and fragmented nuclei seen in the apoptotic cell *(blue arrowheads).* (From Chen D, Yu J, Zhang L. Necroptosis: an alternative cell death program defending against cancer. *Biochim Biophys Acta.* 2016;1865(2):228–236.)

epithelial cells occurs due to ischemic and other cytotoxic insults, whereas inhibition of caspase activity is protective against such injury in cultured and in vivo renal epithelial tubular AKI.[201,202]

Several pathways, including the intrinsic (Bcl-2 family, cytochrome *c*, caspase-9), extrinsic (Fas, FADD, caspase-8), and regulatory (p53 and NF-κB) pathways, appear to be activated during ischemic renal tubular cell injury. It has also been shown that the balance between cell survival and death depends on the relative concentrations of the proapoptotic (Bax, Bcl-2–associated death promoter [Bad], and Bid) and antiapoptotic (Bcl-2 and Bcl-xL) members of the Bcl-2 family of proteins. Overexpression of proapoptotic or relative deficiency of antiapoptotic proteins may lead to the formation of mitochondrial pores. Conversely, the inhibition of such pore formation may occur with the opposite imbalance.[203–205]

Other proteins that have been shown to play a significant role in the apoptotic pathways include NF-κB and p53.[206,207] The central proapoptotic transcription factor p53 can be activated by hypoxia, via HIF-1α, as well as by other noxious stimuli, such as certain drugs (e.g., cisplatin). The kinase-mediated pathways such as ERKs and c-Jun N-terminal kinases (JNKs) are responsible for mediating cellular responses involved in apoptosis, survival, and repair through their interaction with other signals from growth factors, such as hepatocyte growth factor, insulin-like growth factor-1, epidermal growth factor, and vascular endothelial growth factor (VEGF).[208,209] These independent mechanisms can inhibit proapoptotic proteins such as Bad and activate the antiapoptotic transcription of CREB (cyclic adenosine monophosphate response element binding) factors. More recent studies have indicated that there is rapid delivery of small interfering RNA (siRNA) to proximal tubular cells in AKI; targeting siRNA to minimize p53 production leads to a dose-dependent attenuation of apoptotic signaling and kidney function, suggesting potential therapeutic benefit for ischemic and nephrotoxic kidney injury.[210] In vivo, microRNA-24 (miR-24) also regulates the HO-1 and H2A histone family, member X. Overall, these results indicate that miR-24 promotes renal ischemic injury by stimulating apoptosis in endothelial and tubular epithelial cells.[211]

Considering the various pathways available for blockade or modulation, the therapeutic implications of targeting apoptosis in preventing epithelial cell injury are significant. However, it is likely that the "window" to avert lethal injury and prevent cells from progressing to necrosis is in the early initiating apoptotic phases.

Numerous studies have shown that ATP depletion leads to a rise in intracellular calcium through impairment of calcium ATPases, whereas inhibition of Na^+-K^+-ATPase activity potentiates calcium entry into the cell via the sodium-calcium exchanger. Increased cytosolic calcium causes further mitochondrial injury and cytoskeletal alterations.[212] This chain of events results in the downstream activation of proteases such as calpain and phospholipases. Phospholipases such as phospholipase A2 cause direct hydrolytic damage to membranes and also release toxic free fatty acids. They also cause release of eicosanoids that have vasoactive and hemokinetic activities, resulting in an intense surrounding inflammatory response. Calpain mediates plasma membrane permeability and hydrolysis of the cytoskeletal proteins.[213,214] Finally, there is release of lysosomal enzymes and proteases that degrade histones, resulting in accessibility of the endonucleases to the entire segment, typically seen as the smear pattern on gel electrophoresis, in contrast to the typical ladder pattern seen in apoptosis.[215]

Necroptosis. Necroptosis and ferroptosis are two forms of regulated, nonapoptotic cell death. Necrosis is distinguished from apoptosis by the presence of a breakdown of the integrity of the plasma membrane (see Fig. 28.5). As such, necrotic cell death is accompanied by the release of unprocessed intracellular contents, including cellular organelles, highly immunogenic proteins such as ATP, HMGB1, double-stranded DNA, and RNA components, also referred to as damage-associated molecular patterns (DAMPs), a concept known as *necroinflammation.*[216] Although tubular necrosis was thought to be accidental, work done over the last 2 decades has revealed several pathways of genetically determined and regulated necrosis in which receptor interacting serine-threonine protein kinase 1 (RIPK1),[217,218] RIPK3,[219] and its substrate, mixed-lineage kinase domain-like protein (MLKL), have been directly implicated in the regulation of this novel cell death pathway known as necroptosis.[220] The signaling pathway that triggers necroptosis includes the engagement of death receptors in the presence of caspase inhibition, stimulation of Toll-like receptors, signaling through interferons, with the activation of kinase RIPK3, and phosphorylation of pseudokinase MLKL. Phosphorylation of MLKL by RIPK3 leads to a molecular switch mechanism that induces plasma membrane rupture.[221] Demonstration of a protective effect on kidney function with the use of necrostatin-1, an inhibitor of RIPK1, suggests that necroptosis occurs in ischemic AKI.[222] Necroptosis also occurs in the model of cisplatin-induced AKI, as demonstrated by the significant protection of kidney function when using RIPK3 and MLKL-deficient mice.[223]

Ferroptosis. Ferroptosis is a previously unrecognized, non-apoptotic form of regulated cell death that is iron-dependent and characterized by increased lipid peroxidation resulting from lack of activity of the lipid repair enzyme glutathione peroxidase 4 (GPX4).[224–226] This leads to the accumulation of lipid-based ROS, including lipid hydroxyperoxides. This form of iron-dependent cell death is distinct from other forms of cell death such as apoptosis, unregulated necrosis, and necroptosis because it mediates cell death in a noncell autonomous and synchronized manner, providing a potential explanation for nephron loss during AKI.[227] Erastin and RSL3 are ferroptosis-inducing compounds that induce cell death in the absence of apoptotic features.[224,228,229] Erastin and RSL3 induce cell death in the absence of major components of apoptotic machinery, including caspases, BAX, and BAK.[230] In a recent study, ferroptosis was shown to play a key role in folic acid–induced AKI,[231] resulting in increased inflammation. Ferroptosis is also importantly regulated by heme oxygenase-1 (HO-1).[232] Immortalized proximal tubule cells from HO-1$^{+/+}$ mice were much more susceptible to erastin or RSL3 than cells from HO-1$^{-/-}$ mice. Iron supplementation decreased cell viability further in HO-1$^{-/-}$ compared with HO-1$^{+/+}$ cells. Finally, ferrostatin (a ferroptosis inhibitor), deferoxamine (an iron chelator), or *N*-acetyl-L-cysteine (a glutathione replenisher) attenuate erastin-induced ferroptosis[232] (see Fig. 28.4).

Pyroptosis. This highly inflammatory form of regulated cell death requires caspase 1,4/5 (caspase 11 in mice) for activation[197,233] (see Fig. 28.4). Intracellular damage (sterile inflammatory, such as AKI) and release of DAMPs (pathogen-associated molecular patterns [PAMPs]) activate NLRP3 inflammasome and, in turn, lead to caspase activation and cleavage of pro–interleukin-1 β (IL-1β) and pro–IL-18 to their mature forms. In addition, a newly identified pyroptosis executioner, gasdermin D (GSDMD), is activated, releasing the N-terminal fragment (GSDMD-NT).[197,234] The GDSMD-NT fragment is thought to oligomerize and form a membrane pore on the cell membrane,[235] leading to cell swelling, osmotic lysis, and release of IL-1β and IL-18 and other intracellular contents.[197,234] Thus, unlike apoptosis, pyroptosis results in plasma membrane rupture and release of DAMP molecules, leading to the activation of innate immunity and recruitment of immune cells.[236]

Understanding cell death pathways can lead to specific interventions that could involve strategies to preserve apoptosis for avoiding inflammation while blocking the more inflammatory pathways such as pyroptosis, considered the most proinflammatory of the regulated pathways.[197]

Autophagy. Autophagy is an essential mechanism for normal homeostasis, disease pathogenesis, and aging in kidneys.[237–240] Fig. 28.6 reviews the three forms of autophagy—macroautophagy, microautophagy, and chaperone-mediated autophagy:

- Macroautophagy starts with the de novo formation of a cup-shaped isolation double membrane that engulfs a portion of cytoplasm.

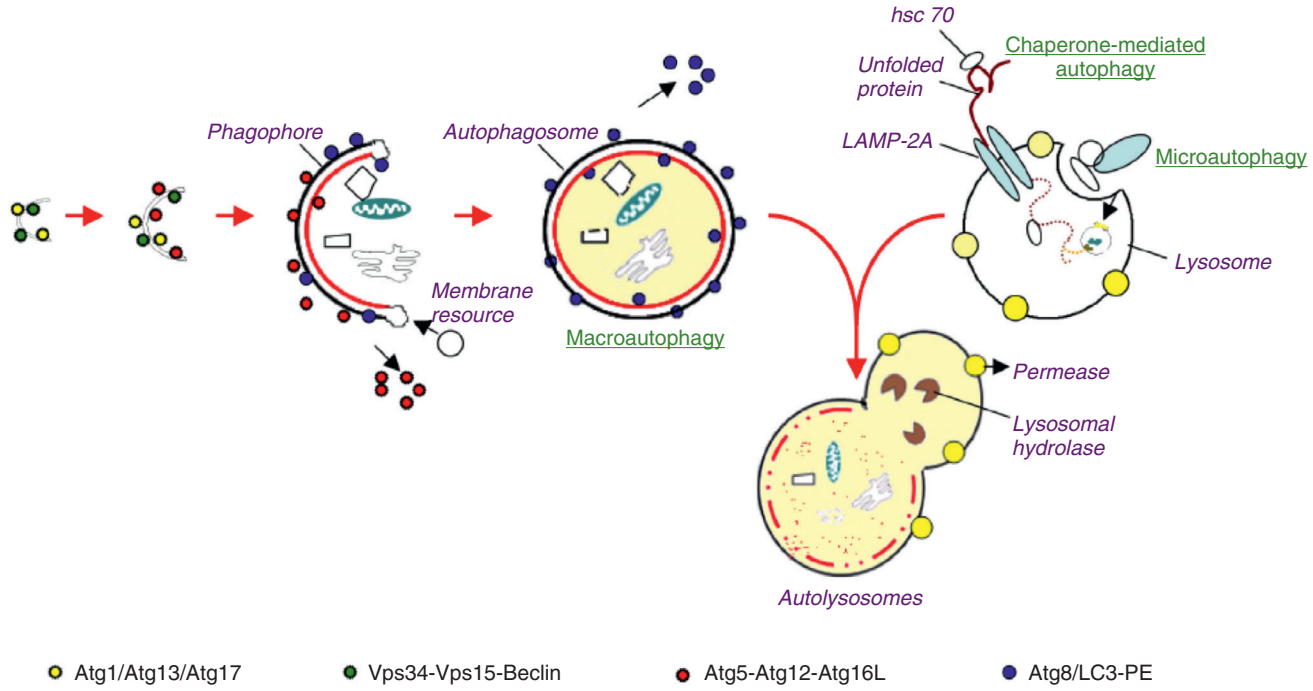

Fig. 28.6 Autophagy. The forms of autophagy are macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy starts with the de novo formation of a cup-shaped isolation double membrane that engulfs a portion of cytoplasm. Microautophagy involves the engulfment of cytoplasm instantly at the lysosomal membrane by invagination, protrusion, and separation. Chaperone-mediated autophagy is a process of direct transport of unfolded proteins via the lysosomal chaperonin hsc70 and LAMP-2A. All forms of autophagy subsequently lead to the degradation of intraautophagosomal components by lysosomal hydrolases. *PE,* Phosphatidylethanolamine. (From Periyasamy-Thandavan S, Jiang M, Schoenlein P, Dong Z. Autophagy: molecular machinery, regulation, and implications for renal pathophysiology. *Am J Physiol Renal Physiol.* 2009;297(2):F244–F256.)

- Microautophagy involves the engulfment of cytoplasm instantly at the lysosomal membrane by invagination, protrusion, and separation.
- Chaperone-mediated autophagy is a process of direct transport of unfolded proteins via the lysosomal chaperonin hsc70 and the receptor for chaperone-mediated autophagy, LAMP-2A.

Autophagy is highly conserved and is a lysosomal degradation pathway for the salvation and reuse of degraded proteins, lipids, and organelles to generate basic structural components and energy. It also removes obsolete and dysfunctional organelles and cytotoxic protein aggregates for cellular homeostasis. Under pathogenic conditions, autophagy plays a key role in ischemic, toxic, immunologic, and oxidative insults that lead to the induction of autophagy in renal epithelial cells, which plays a key role in altering the course of disease.[238] Autophagy-mediated leukocyte clearance is an important mechanism for resolving inflammation; it is a function performed by macrophages.[239] Autophagy is characterized by the formation of autophagosomes, a double-membrane vesicles wrapping cytosolic components and organelles that are destined for degradation. Autophagosomes fuse with lysosomes, the contents are degraded, and the products are salvaged and serve as building blocks for cellular function.[237] There are a number of genes and proteins associated in autophagy (autophagy-related; Atg).[237]

Autophagy serves a protective function in renal tubular cells during ischemia-reperfusion injury; it precedes the appearance of apoptotic cells, and suppression of autophagy exacerbates renal injury, in part by inhibiting apoptosis.[241,242] The importance of autophagy has been demonstrated through the generation of proximal tubule-specific ATG5 or ATG7 knockout mice.[243–245] The absence of ATG5 in proximal tubules led to the accumulation of deformed mitochondria and cytoplasmic inclusions or protein aggregates.[243] The lack of ATG5 in distal tubules did not cause significant alterations in kidney function.[244] Similar results were observed in proximal tubule-specific ATG7 knockout mice.[246] Importantly, renal ischemia-reperfusion injury was exaggerated in proximal tubule-specific ATG5 or ATG7 knockout mice.[243,244,246] Autophagy activation was also detected in cisplatin-induced AKI in mice.[247] ATG7 knockout mice demonstrated worse kidney function and injury with cisplatin treatment.[246] Chloroquine, an inhibitor of autophagy, tested in a cisplatin-induced model of AKI, blocked the lysosomal degradation of LC3 in autophagosomes and worsened kidney function.[246] Similarly, autophagy was induced in the septic AKI model of LPS treatment of mice, and inhibition by chloroquine or tubular ATG7 ablation worsened LPS-induced AKI.[248] Thus, data from pharmacologic studies and genetically deficient mice have provided strong evidence for a renoprotective role of autophagy. In addition, dysregulated autophagy can result in increased renal degeneration, leading to progressive kidney disease, as characterized by interstitial fibrosis.[249,250]

NETosis. NETosis is a specific form of regulated neutrophil death in which neutrophils release neutrophil extracellular traps (NETs), which are extracellular structures composed of chromatin and histones that enable immobilization and killing of bacteria.[251] On their activation by IL-8, LPS, bacteria or activated platelets, neutrophils initiate a program that leads to their death and the formation of NETs.[252] The activation pathways are known to involve Toll-like receptors (TLRs), cytokines, and Fc receptors.[251,253] This form of cell death is different from apoptosis and necrosis. Cell death is independent of caspases and is not accompanied by fragmentation.[252,254]

Infiltrating neutrophils undergoing NETosis contribute further to organ damage in ischemic AKI. Using the IRI model, Nakazawa and coworkers have demonstrated that infiltrating neutrophils undergo NETosis, leading to the release of cytotoxic DAMPs, such as histones, which exacerbate tubular epithelial cell injury and interstitial inflammation.[255] Using an inhibitor of peptidyl arginine deiminase (PAD), a key enzyme in NET formation, or depleting neutrophils with anti-Ly6G mab, reduced NET formation, tissue necrosis, and biomarkers of AKI and improved kidney function. Furthermore, the authors also demonstrated the presence of NETs in kidney biopsies of patients with ATN.

Innate Immunity and Inflammation

Kidney Microenvironment. The interstitial microenvironment, between the basement membranes of epithelial cells and peritubular capillaries, is a reactive compartment containing mononuclear phagocytes, interstitial fibroblasts, and pericytes, as well as various soluble mediators.[256] The mononuclear phagocytic system consists of bone marrow-derived macrophages and dendritic cells,[257] which overlap in functional characteristics and surface biomarkers.[258] Macrophages are resident tissue phagocytic cells that function generally to clear dying cells and produce cytokines and growth factors.[259,260] Although this system is well known to be activated in response to foreign pathogens,[261] the microenvironment responds to endogenous molecules released from dying cells following tissue injury or to changes produced by conditions such as hypoxia, ischemia, or other forms of sterile inflammation. Matzinger has proposed the danger model to explain these exceptions to the classical role of immune responsiveness to foreign antigen.[262] Dendritic cells are activated by DAMPs or PAMPs[263] and are the key initiators of the innate immune system, following ischemia-reperfusion (Fig. 28.7).

Mononuclear Phagocytes in Acute Kidney Injury. Dendritic cells, a resident population of bone marrow–derived cells, and macrophages form a network between the basement membranes of tubular epithelia and peritubular endothelial cells.[256,264] Although dendritic cells and macrophages are often considered as distinct cell types with characteristic functions, recent studies have shown considerable overlap in cell surface markers and function between dendritic cells and macrophages[257] (Fig. 28.8). Located in the interstitial space, dendritic cells have access to endogenous and exogenous DAMPs and PAMPs released by epithelial cells, invading organisms, and infiltrating cells, and thus are key initiators, potentiators, and effectors of the innate immune system.[263,265]

Dendritic cells have enormous plasticity and can be antiinflammatory or proinflammatory.[266,267] Dendritic cells contribute early in the course of IRI-induced activation of NKT cells and the IL-17/IL-23 signaling pathway.[268,269] The importance of dendritic cells in activating the innate immune response in AKI has been determined through depletion of dendritic cells. This was accomplished using transgenic mice expressing the human diphtheria toxin receptor ([DTR]; human heparin-binding epidermal growth factor-like growth

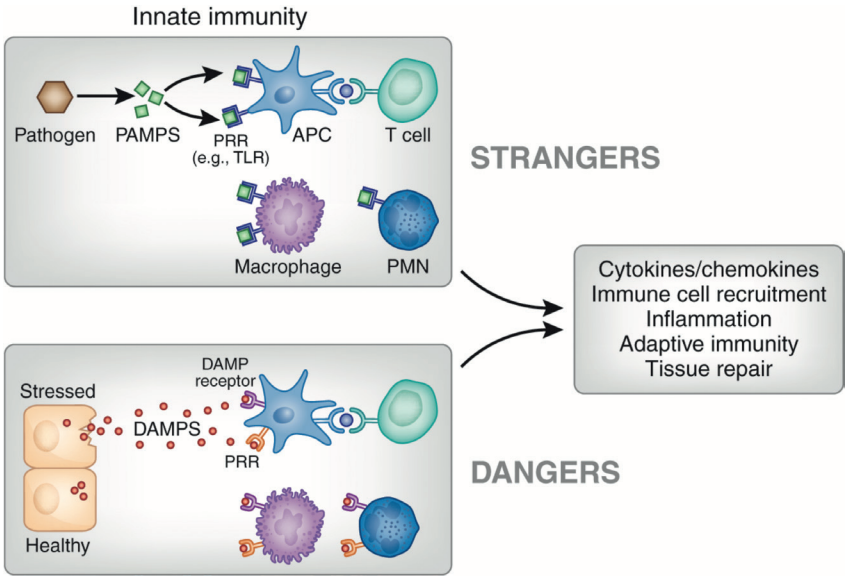

Fig. 28.7 Danger and stranger models. Infections of pathogenic bacteria or viruses cause the release of pathogen-associated molecular patterns *(PAMPs)* that bind to pattern recognition receptors *(PRRs)*, such as Toll-like receptors *(TLRs)*, on immune cells and stimulate an innate immune response that is accompanied by inflammation and activation of adaptive immunity, and eventually processes to resolve the infection and allow for tissue repair. The danger model recognizes that similar events occur when cells are stressed or injured and that necrotic cells release molecules normally hidden within the cell. In the extracellular space, these damage-associated molecular patterns *(DAMPs)* can bind to PRRs or to specialized DAMP receptors to elicit an immune response by promoting the release of proinflammatory mediators and recruiting immune cells to infiltrate the tissue. The immune cells that participate in these processes include, for example, antigen-presenting cells *(APCs)*, such as dendritic cells and macrophages, as well as T cells and neutrophils (polymorphonuclear leukocytes *[PMNs]*). DAMPs may also stimulate adaptive immunity and participate in autoimmune responses and tissue repair. A wide variety of intracellular and extracellular molecules function as DAMPs when released from cells (see Table 28.1). The functions of such a diverse group of molecules may not yet be fully elucidated; it is unknown whether different DAMPs have specific roles, whether specific functions are elicited in different cell types or conditions, or even whether immune responses to DAMPs can be distinguished from those of PAMPs. (From Rosin DL, Okusa MD. Dangers within: DAMP responses to damage and cell death in kidney disease. *J Am Soc Nephrol.* 2011;22(3):416-425.)

factor in $CD11c^+$ cells (CD11c-DTR mouse).[270] Using diphtheria toxin (DT) and depleting kidney $CD11c^+$ dendritic cells prior to IRI, there was significantly less injury in DT-treated CD11c-DTR mice compared with CD11c DTR mice treated with a catalytically inactive mutant DT (mDT),[271] strongly supporting the concept that dendritic cells contribute to the early innate response in IRI. Depletion of dendritic cells reduced IRI, and deletion of dendritic cell sphingosine 1-phosphate receptor 3 ($S1P_3R$) or inhibition of $S1P_2R$ resulted in protection from IRI.[271–273] They are known to be the earliest producers of IL-6, tumor necrosis factor-α (TNF-α), MCP-1, and RANTES (regulated on activation, normal T cell–expressed and secreted).[274] In addition to initiating the recruitment of inflammatory cells, dendritic cells also participate in recovery via IL-10 production.[275] Dendritic cells can also induce tolerance. Tolerogenic dendritic cells are functionally immature, express inadequate positive or enhanced negative costimulatory signals and reduced proinflammatory cytokines, and can generate immune tolerance by inducing T cell anergy or deletion or by induction or expansion of regulatory T (Treg) cells.[276,277] Adenosine 2A receptor (A_2AR)-induced tolerized dendritic cells, suppressed NKT cell activation in vivo and attenuated kidney IRI.[278] However, mature dendritic cells also can promote tolerance.[276,279] Both immature and mature dendritic cells can prime Treg cells that prevent autoimmunity. In contrast to IRI, dendritic cell ablation in DTR mice increased injury in a cisplatin-induced model of AKI, which demonstrates the importance of dendritic cells for tissue protection in a different model of kidney injury.[280] Thus, these studies have revealed disparate roles of tissue resident dendritic cells in AKI and suggest that the differing interstitial microenvironment created by different pathogenic circumstances, such as cisplatin toxicity, IRI, or endogenous molecules (e.g., PAMPS, DAMPS, cytokines, autacoids such as adenosine) may regulate dendritic function.[266] Finally, dendritic cells migrate away from the kidney via the lymphatic system to present antigen and regulate lymphocytic responses.[281] Thus, dendritic cells serve at the crossroads of communication between the epithelium and endothelium, regulating both innate and adaptive immunity, self-tolerance, and tissue injury and repair.

Macrophages are phagocytic innate immune cells that contribute to host defense and, based on surface markers, five distinct populations have been identified.[282,283] Tissue-resident macrophages are derived from a heterogeneous population of bone marrow derived-monocytes.[260,284–286] They are characterized by low surface expression of chemokine receptor 2 (CCR2), GR-1, and Ly6C and high surface expression of the fractalkine receptor CX3CR1.[260,287] These monocytes migrate into normal tissue and differentiate into resident dendritic cells and macrophages.[286] In contrast, macrophages that infiltrate into inflamed tissue have a phenotype characterized by high surface expression of CCR2, Ly6C, and GR-1 and low surface expression of CX3CR1. It is likely that the

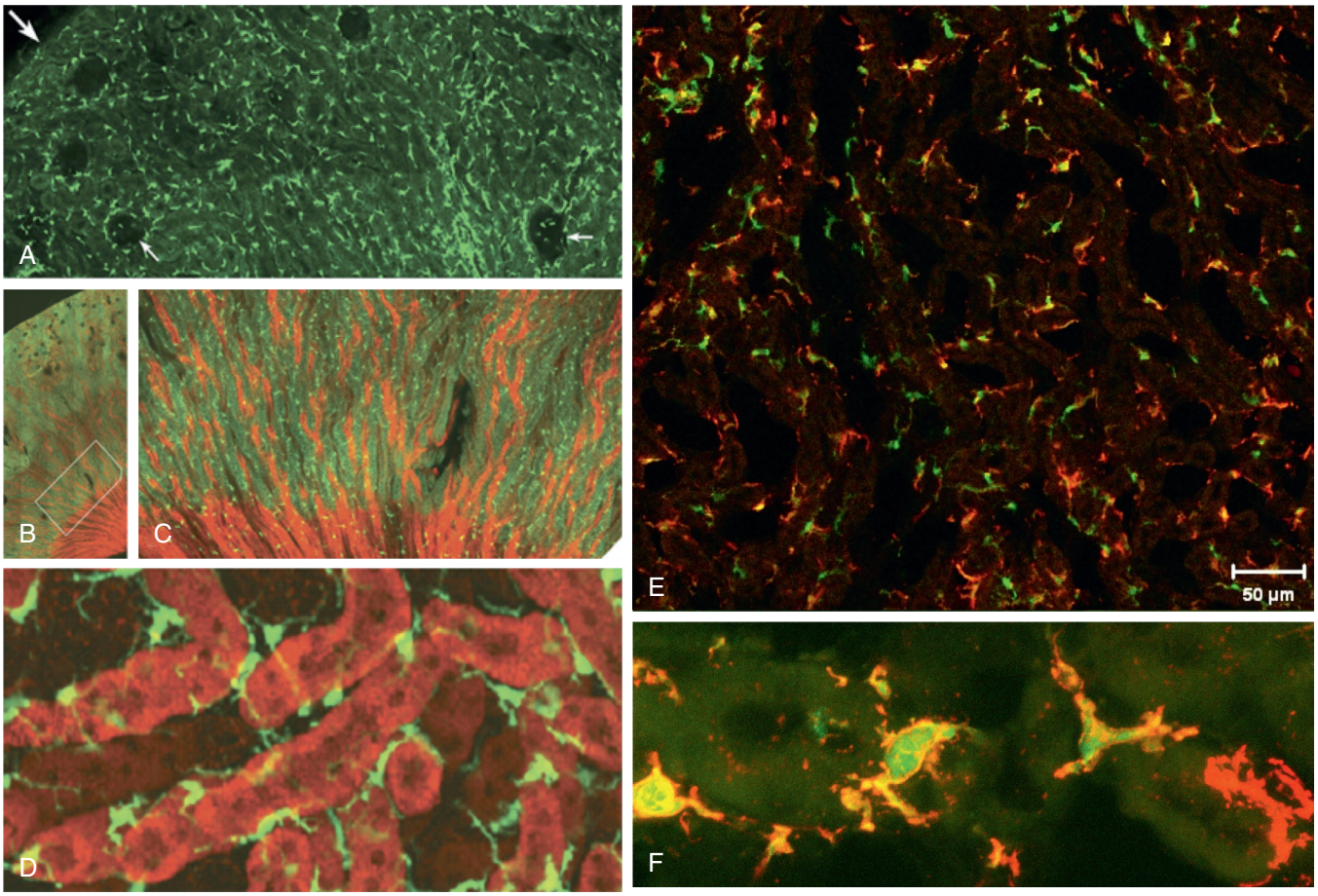

Fig. 28.8 Heterogeneity of mononuclear phagocytes within the microenvironment. Mononuclear phagocytes within the microenvironment, identified as CX3CR1^{+}/GFP^{+} cells (labelled *green*), in the kidney are shown in panels A to D, and the heterogeneity of mononuclear phagocytes is shown in panel E. (A) A heterogenous population of CX3CR1^{+}/GFP^{+} mononuclear phagocytes in superficial cortex includes dendritic cells and macrophages and reside from the cortex and medulla. CX3CR1^{+} GFP^{+} cells extend to the edge of the cortex *(large arrow)* and populate the entire interstitial space abundantly. Bowman's capsule is encased by CX3CR1^{+}/GFP^{+} cells *(small arrows).* Also notable are CX3CR1^{+} GFP^{+} cells that lie within each glomeruli. (B) Rhodamine-conjugated peanut agglutinin *(red fluorescence)* highlights distal tubules and collecting ducts, capsule *(upper left)* to the papilla *(lower right).* (C) Magnification of the boxed area in panel B showing CX3CR1^{+} GFP^{+} cells in the interstitium of the medulla, including transition into pyramidal tracks. Note the same spatial regularity as in the cortex. (D) Three-dimensional rendering of stellate-shaped CX3CR1^{+} GFP^{+} cells within the interstitium demarcated by tubular segments in the medulla *(red fluorescence).* (E) Kidney sections from the medulla of CX3CR1^{+}/GFP^{+} mice. GFP is expressed mainly on monocyte-macrophages and dendritic cells, and many CX3CR1^{+}GFP^{+} green fluorescing cells are seen in the cortex; most IA^{+} cells *(red label)* are seen in the medulla. Apparent in this image is the heterogeneity of CX3CR1^{+}/GFP^{+}–only cells, IA^{+}–only cells, and dual labeling with CX3CR1^{+}/GFP^{+} and IA^{+}, which in the latter case represents dendritic cells in the medulla. (F) Higher magnification, Z-stack projection image of five optical slides at 0.69-mm intervals. These results demonstrate the heterogeneity of mononuclear phagocytes within the kidney microenvironment. (A–D from Soos TJ, Sims TN, Barisoni L, et al. CX3CR1+ interstitial dendritic cells form a contiguous network throughout the entire kidney. *Kidney Int*. 2006;70(3):591–596; E, F from Li L, Huang L, Sung SS, et al. The chemokine receptors CCR2 and CX3CR1 mediate monocyte/macrophage trafficking in kidney ischemia-reperfusion injury. *Kidney Int.* 2008;74(12):1526–1537.)

microenvironment in the tissue determines their phenotype. TNF-α, IL-4, and IL-15 skew monocyte differentiation toward a dendritic cell phenotype,[288–290] whereas interferon-gamma (IFN-γ) and IL-6 direct monocyte differentiation toward a macrophage phenotype.[291,292] Following kidney IRI, through monocyte chemoattractant protein 1 (MCP-1/CCL2) signaling via monocyte CCR2 receptors,[293] monocytes migrate into inflamed kidneys within 24 hours.[269,271]

Although controversial, and challenged by recent studies, the original classification of M1 and M2 macrophages does provide an important functional framework.[294] M1 macrophages, also referred to as "classic macrophages," are activated by IFN-γ and LPS and express high levels of inducible nitric oxide synthase (iNOS). M1 macrophages have high microbicidal activity through the production of proinflammatory cytokines such as TNF-α, IL-6, and IL-12 via signal transducer and activator of transcription 1 (STAT1), NF-κB, and ROS.[283] M2 macrophages, referred to as "alternative macrophages," are activated by IL-4 or IL-13 and participate in tissue repair and resolution of inflammation through insulin-like growth factor 1 (IGF-1), mannose receptor 1 (Mrc1/CD206), and arginase-1 (Arg1) following STAT6 activation.[295]

Evidence for these disparate roles played by macrophages can be assessed in vivo through the administration of liposomal clodronate. Liposomal clodronate is engulfed by macrophages

and clodronate is released from the liposome through action of lysosomal phospholipases in the macrophage, becomes toxic, and kills cells by apoptosis.[296] The functional role of macrophages was determined by liposomal clodronate depletion studies that focused on the time course for macrophage depletion (early vs. late). Depletion of kidney and spleen macrophages using liposomal clodronate before renal IRI prevented AKI, and adoptive transfer of macrophages reconstituted AKI.[297] However, depletion of macrophages 3 to 5 days after IRI slowed tubular cell proliferation and repair,[283] suggesting that M1 macrophages exhibiting a proinflammatory phenotype are important for injury, whereas M2 macrophages exhibiting an antiinflammatory phenotype are important for tissue repair.[283,298,299]

Following IRI, the proinflammatory monocyte entering into the kidney tissue is activated to a proinflammatory macrophage through the infiltration of polymorphonuclear leukocytes, T cells, and NKT cells.[268,300,301] Resident dendritic cells activate NKT cells to produce INF-γ and other inflammatory mediators such as ROS, semaphorin-3A, DAMPs, and TNF-α, which conditions the microenvironment and favors the proinflammatory macrophage phenotype.[283] These proinflammatory macrophages then produce proinflammatory cytokines, which lead to further tissue injury. Whether polarization of macrophages from M1 to M2 involves the recruitment of circulating cells or reprogramming is unclear.[283,302] Studies by Lee and colleagues have supported the concept that macrophage polarization occurs in situ.[303] In these studies, the investigators injected labeled M1 macrophages at the time of injury and examined labeled macrophages that infiltrated the injured kidneys. Most of the labeled cells maintained an M1 phenotype; however, when labeled M1 macrophages were injected 3 days after injury, most of the macrophages expressed an M2 phenotype. These studies support the concept that macrophages may be reprogrammed in situ,[283,302,304] and that the kidney microenvironment provides important conditioning cues.[266]

Early inflammation is classically characterized by the margination of leukocytes to the activated vascular endothelium via interactions between selectins and ligands that allows firm adhesion, followed by transmigration.[305,306] A number of potent mediators are generated by the injured proximal tubular epithelial cell, including proinflammatory cytokines such as TNF-α, IL-6, IL-1β, MCP-1, IL-8, transforming growth factor-β (TGF-β), and RANTES.[278,307] TLR2 is an important mediator of endothelial ischemic injury, and TLR4 has been shown to play a similar role in animal models of both ischemic and septic injury,[308] especially in PTC.[309]

Polymorphonuclear Leukocytes in Acute Kidney Injury. Neutrophils accumulate early in ischemic injury in animal models.[271] Both IL-17A and IFN-γ were shown to be produced by neutrophils and may positively regulate neutrophil transmigration to the injured kidney following kidney IRI[268] (Fig. 28.9). Although neutrophils are seen early in rodent models of AKI, whether they play a pathogenic role remains controversial because inhibition or depletion studies of neutrophils leads to protection[305,310,311] or lack of protection.[312] Blockade of neutrophil function or neutrophil depletion has been shown to provide only partial protection against injury.

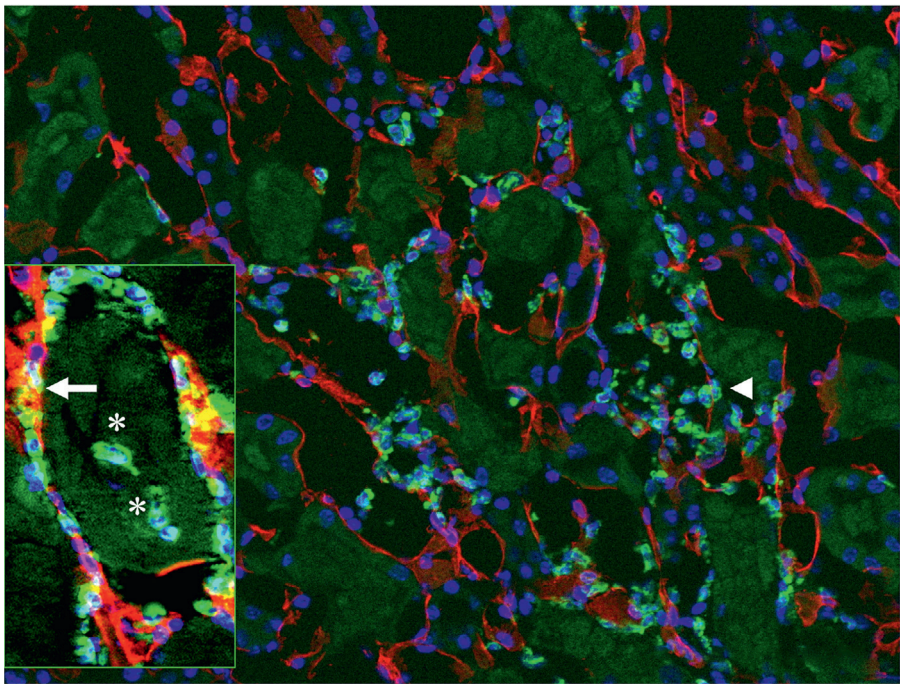

Fig. 28.9 Localization of neutrophils to interstitial and marginated compartments in the kidney. Immunofluorescence staining of kidney outer medulla using antibodies to 7/4 (*green*, neutrophils) and CD31 (*red,* vascular endothelium). Nuclei are depicted by DAPI labeling *(blue).* Neutrophils in both sides of the vascular endothelial wall. *Inset,* Z-stack image (7.0 μm) of 12 optical slices of the kidney at 0.6-μm intervals. Shown are kidney neutrophils in the interstitium and peritubular capillaries *(arrows)*; neutrophils that have transmigrated into the lumen of the tubule *(*)* are shown in the inset. (From Awad, AS, Rouse, M, Huang, L, et al. Compartmentalization of neutrophils in the kidney and lung following acute ischemic kidney injury. *Kidney Int.* 2009;75:689–698.)

Vascular adhesion protein-1 (VAP-1) is an adhesion molecule[313,314] associated with inflammatory conditions. Tanaka and colleagues have found that VAP-1 is expressed primarily in pericytes, and a specific VAP-1 inhibitor, RTU-1096, attenuated renal IRI and decreased neutrophil infiltration. The protective effect of VAP-1 inhibition was absent in neutrophil-depleted rats, suggesting an important role of neutrophil infiltration.[315]

Other leukocytes, including but not limited to macrophages, NKT cells, B lymphocytes, and T lymphocytes contribute to kidney IRI.[297,301,316–319] Selective depletion, knockout mice models, and specific blockade have shown that all these cells do mediate tubular injury at various phases and that there are synergistic interactions between different cellular types.[297,316–319]

Whether released from the endothelium or the epithelial cell, numerous cytokines exert a concerted biochemical effort to augment the inflammatory response seen as a result of ischemic or septic injury.[317] Furthermore, mouse tubular cells when stimulated with LPS in culture, upregulate TLR2, TLR3, and TLR4 and secrete CC-chemokines such as CC motif chemokine ligand 2 (CCL2)/MCP-1 and CCL5/RANTES. These data suggest that tubular TLR expression might be involved in mediating interstitial leukocyte infiltration and tubular injury during bacterial sepsis.[320]

TLR2 and TLR4 are constitutively expressed on renal epithelium, and their expression is enhanced following renal IRI. El-Achkar and coworkers have shown that in a CLP rat model of sepsis, TLR4 expression increases markedly in all tubules (proximal and distal), glomeruli, and the renal vasculature.[321] Genetic deletion of TLR2 or TLR4 protects from renal IRI[309,322] and from cisplatin-induced AKI. In this later study, Zhang et al.,[323] using bone marrow chimeras, found that renal parenchymal TLR4, rather than hematopoietic-derived TLR4, mediated cisplatin-induced nephrotoxicity. These studies are consistent with studies by Wu and coworkers[309] and clearly demonstrate the important role TLR plays in AKI.

T Cells in Acute Kidney Injury. Early work by Burne-Taney and coworkers has demonstrated that T lymphocytes contribute importantly to renal IRI.[312,324,325] However, conventional $CD4^+$ T cells are thought to play an obligatory role in antigen-specific, cognate immunity that requires 2 to 4 days for T cell processing, a time course that cannot explain the rapid, innate immune response following IRI. Natural killer T (NKT) cells are a T cell sublineage[326] and, in mice, NKT cells express an invariant T cell receptor TCRα chain, Vα14-J18. In contrast to conventional T cells, the NKT cell TCR does not interact with peptide antigen presented by classic major histocompatibility complex (MHC) class I or II; rather, it recognizes glycolipids presented by the class I-like molecule, CD1d. On NKT cell activation, vigorous cytokine secretion occurs within 1 to 2 hours, including Th1-type (IFN-γ, TNF) and Th2-type (IL-4, IL-13) cytokines at the same time.[326–332] The rapid response by NKT cells following activation can amplify and regulate the function of dendritic cells, Treg cells, NK and B cells, and conventional T cells and thus link innate and adaptive immunity.[333–338] In AKI, NKT cells participate in early innate immune response to kidney IRI.[301] NKT-produced IFN-γ was found as early as 3 hours following IRI,[301] supporting previous work demonstrating that $CD4^+$ T cell IFN-γ production is responsible for kidney injury.[339,340] These data demonstrate the central role of IFN-γ from $CD4^+$ T and/or NKT cells in the pathogenesis of renal IRI.

Treg cells[318,319] have also recently been shown to play a role in ischemic AKI.[341] Liu and colleagues have shown that in a murine model of ischemic AKI, there is significant trafficking of Treg cells into the kidneys after 3 and 10 days.[342] Postischemic kidneys had increased numbers of TCR-β^+–$CD4^+$ and TCR-β^+–$CD8^+$ T cells, with enhanced proinflammatory cytokine production. These investigators also noted that Treg depletion starting 1 day after ischemic injury using anti-CD25 antibodies increases renal tubular damage, reduces tubular proliferation at both time points, and enhances infiltrating T lymphocyte cytokine production at 3 days and TNF-α generation by TCR-β^+–$CD4^+$ T cells at 10 days. In separate mouse studies, infusion of $CD4^+$–$CD25^+$ Treg cells 1 day after initial injury reduced IFN-γ production by TCR-β^+– $D4^+$ T cells at 3 days, improved repair, and reduced cytokine generation at 10 days. These studies demonstrate that Treg cells infiltrate post-IRI kidneys during the healing process and promote repair, likely through modulation of proinflammatory cytokine production of other T cell subsets.[342]

The role of Treg cells has been further extended by Kinsey and coworkers, who have shown that partial depletion of Treg cells with an anti-CD25 monoclonal antibody potentiates kidney damage induced by IRI and that reducing the number of Treg cells results in more neutrophils, macrophages, and innate cytokine transcription in the kidney after IRI.[343] Furthermore, FoxP3 (forkhead box P3)$^+$ Treg cell–deficient mice accumulated a higher number of inflammatory leukocytes after renal IRI than mice containing Treg cells, and that cotransfer of isolated Treg cells and Scurfy lymph node cells significantly attenuated IRI-induced renal injury and leukocyte accumulation.[343] Studies of adoptively transferred Treg cells have shown that IL-10 production, adenosine production through CD73, expression of the adenosine 2A receptor, and programmed cell death 1 (PD-1) on the cell surface are required by Treg cells to protect recipient mice from IRI.[319] Studies have demonstrated that PD-1, a negative costimulatory molecule expressed on T lymphocytes, monocytes, dendritic cells, and B cells,[344,345] is indispensable for Treg function. Administration of monoclonal antibodies to PD-1 or a genetic deficiency of PD-1 on Tregs exacerbated impaired kidney function and ATN after subthreshold ischemia.[346] Stremska and coworkers have synthesized a hybrid cytokine, IL233, which combines IL-2 and IL-33.[347] Tregs require IL-2 for homeostasis and upregulate IL-33, which promotes the recruitment and activation of innate lymphoid cells (ILC2s).[348] Administration of the novel hybrid cytokine, IL233, increased endogenous Tregs in blood and spleen and prevented IRI more efficiently than a mixture of IL-2 and IL-33 administered separately. IL233 also increased the proportion of ILC2s in blood and kidneys, and adoptive transfer of ILC2s protected mice from IRI.[347] Thus, the many barriers to cell-based therapy may be overcome through this hybrid cytokine, which increases endogenous Tregs. Rapid translation to human studies would be a major advancement in the field.

B Lymphocytes. Studies to evaluate the role of B cells and the B1 subset on ischemia-induced AKI have remained controversial, with some studies showing that B cells may be deleterious. Burne-Taney and associates have shown that μMT

mice, which lack B cells and all immunoglobulins, are protected from AKI despite similar levels of infiltrating granulocytes and macrophages in the postischemic kidneys of wild type (WT) mice.[316] These investigators demonstrated that μMT mice were rendered sensitive to ischemia-induced AKI after replenishing these mice with WT serum, but not B cells, thus indicating that a serum factor enhanced the cytotoxic effect of infiltrating granulocytes and macrophages on the ischemic tubular cells. However, Renner and colleagues, using the same μMT mice, could not show that these mice were protected from ischemia-induced AKI.[349] In fact, these mice had more severe injury when compared with control WT mice. Additionally, Lobo and associates failed to show protection from ischemia-induced AKI when WT mice were acutely depleted of B cells (with anti-CD20) and not immunoglobulins.[350] More studies are needed to study the role of B cells and immunoglobulins in AKI; it may be preferable to deplete B cells or subsets of B cells acutely to study their role. μMT mice have other immune deficiencies, including low Tregs and lack of TcR diversification, which may have contributed to more severe injury in the experiments by Thurman and colleagues.[351,352]

The role of the B1 subset of B cells in murine models of ischemia has also been controversial. Ray, Zhang, and associates have clearly shown that natural IgM produced by B1 cells is deleterious in ischemia-induced injury of murine skeletal muscle, cardiac muscle, and bowel.[352–354] They demonstrated that ischemia exposed nonmuscle myosin, heavy-chain type IIA and C (NMM) neoantigen in these organs and, by immunohistochemistry, they clearly demonstrated binding of natural IgM and complement to NMM in these ischemic organs. Furthermore, they showed that natural IgM was pathogenic because these same organs were resistant to ischemic injury in immunoglobulin-deficient $Rag1^{-/-}$ mice, but become susceptible to ischemia if these mice are replenished with purified IgM. On the other hand, Lobo and colleagues have shown that natural IgM protects WT kidneys from ischemic AKI by inhibiting the innate inflammation that occurs after ischemic injury.[355] Both they and others have clearly shown that $Rag1^{-/-}$ mice are also susceptible to ischemic AKI in the absence of IgM.[350] Kidneys from IgM knockout mice, deficient in only IgM but not B cells and other immunoglobulins or Tregs, are very sensitive to minimal ischemia that is insufficient to cause AKI in WT mice.[355] Additionally, Renner and associates could not show binding of IgM to ischemic tubules or peritubular capillaries in WT mice. It is therefore possible that NMM, which is also present in the kidney, is not exposed after ischemic injury.[349] Such observations also demonstrate that the mechanisms that cause ischemic injury in the kidney may be different from those of other organs.

Inflammation

Altered endothelial cell function also mediates inflammation, a hallmark of ischemic injury that has been the subject of numerous studies. Ischemia induces the increased expression of a number of leukocyte adhesion molecules, such as P-selectin, E-selectin, and intercellular adhesion molecule (ICAM) and B7-1. Consequently, it has been shown that strategies to block pharmacologically or genetically ablate the expression of these molecules are protective against ischemic or septic AKI.[311] Investigators have also shown that T cells play a major role in vascular permeability during ischemic injury. Gene microarray analysis has shown that the production of TNF-α and IFN-γ protein is increased in CD3 and CD4 T cells from the blood and kidney after ischemia. Furthermore, it has also been demonstrated that in CD3, CD4, and CD8 T cell–deficient mice, there is a significantly attenuated rise in renal vascular permeability after ischemic injury. Hence, T cells directly contribute to the increased vascular permeability, potentially through T cell cytokine production.[342,356] Another feature noted during inflammation and endothelial cell injury is the phenomenon of erythrocyte trapping with rouleaux formation, prolonging the reduction in renal blood flow and exacerbating tubular injury.[357] Studies have also shown a role of the sphingosine 1-phosphate receptor (S1PR) in maintaining structural integrity after AKI. Thurman and coworkers have shown that S1PRs in the proximal tubule are necessary for stress-induced cell survival, and S1PR agonists are renoprotective via direct effects on tubular cells.[358,359]

DNA microarray analysis of ischemic kidneys from TLR4-sufficient and TLR4-deficient mice has shown that pentraxin 3 (PTX3), an endothelial induced protein, is upregulated only in TLR4-sufficient mice. Transgenic knockout of PTX3 ameliorated AKI. PTX3 was shown to be expressed predominantly on the peritubular endothelia of the outer medulla of the kidney in control mice and AKI increased PTX3 protein in the kidney and the plasma. Stimulation studies performed in primary renal endothelial cells have suggested that endothelial PTX3 is induced by pathways involving TLR4 and ROS and demonstrated that these effects could be inhibited by conditional endothelial knockout of MyD88. Compared with WT mice, PTX3 knockout mice had decreased endothelial expression of cell adhesion molecules at 4 hours of reperfusion, possibly contributing to a decreased early maladaptive inflammation in the kidneys of knockout mice, whereas later, at 24 hours of reperfusion, PTX3 knockout increased the expression of endothelial adhesion molecules when regulatory and reparative leukocytes enter the kidney. Thus, endothelial PTX3 plays a pivotal role in the pathogenesis of ischemic AKI.[360]

The role of glomerular endothelial injury in AKI is unclear. Studies in a mouse model of LPS-induced sepsis have shown decreased abundance of endothelial surface layer heparan sulfate proteoglycans and sialic acid, leading to albuminuria, likely reflecting altered glomerular filtration permselectivity and decreased expression of VEGF. LPS treatment also decreased the GFR, caused ultrastructural alterations in the glomerular endothelium, and lowered the density of glomerular endothelial cell fenestrae. These LPS-induced effects were diminished in TNF receptor 1 (TNFR1) knockout mice, suggesting the role of TNF-α activation of TNFR1, and intravenous administration of TNF also led to a decreased GFR and loss of glomerular endothelial cell fenestrae, increased fenestrae diameter, and damage to the glomerular endothelial surface layer. Thus, glomerular endothelial injury, mediated by higher TNF-α and lower VEGF levels, extends the development and progression of AKI and albuminuria in the LPS model of sepsis in the mouse.[361]

In addition to immune cells responding and sensing DAMPs and PAMPs (see later), proximal tubule cells (PTCs) function as a sensor of both self and nonself; DAMPs and PAMPs serve as recognition signals for pattern recognition receptors (PRRs)

such as TLR4.[362] Proximal tubule TLR4 is upregulated and migrates to the apical domain in response to LPS in S1 PTCs, which are the earliest segments of epithelial cell postglomerular filtration.[321] Interestingly, the S1 cell internalizes and processes LPS via TLR4 receptors, which is inducible with preexposure to LPS but is protected from injury by upregulated defense mechanisms, including heme oxygenase-1 (HO-1) and sirtuin 1 (SIRT1), two cytoprotective proteins. However, S2 to S3 PTCs undergo oxidative injury with minimal uptake of LPS, implying communication, crosstalk, and coregulation between the segments following LPS exposure.[363] This injury is dependent on CD14, likely due to peroxisomal disruption, perhaps mediated by TNF-α, and the PTC injury was found to be independent of systemic cytokines.

Another role for epithelial TLR4 and MyD88 in mediating ischemic injury has been shown by Wu and colleagues.[309] In addition, in these studies, the relative contribution of epithelial versus hematopoietic TLR4 to kidney damage following IRI was assessed using bone marrow chimeras in which $TLR4^{-/-}$ mice were engrafted with WT hematopoietic cells (and vice versa). Both hematopoietic and parenchymal TLR4 contributed to kidney injury, although the effect was more pronounced when TLR4 was expressed only on parenchymal cells. These results suggest that TLR4 signaling on hematopoietic and intrinsic kidney cells contributes to mediating kidney damage but a more significant role is played by intrinsic kidney cells. Similar results with TLR2 knockout mice and chimeric mice have suggested that epithelial TLR2 plays a prominent role during ischemic injury.[364] Cytokine and chemokine production was reduced and white blood cell (WBC) infiltration was minimized in chimeric mice using antisense therapy. These studies suggest that renal-associated TLR2 is an important initiator of inflammatory responses that lead to renal injury.

Cytokines and chemokines released by PTCs in response to cell injury have direct effects on endothelial function. Using two-photon microscopy, investigators recorded cellular and physiologic responses to fluorescent cytopathologic *Escherichia coli* microinjected into early proximal tubule segments.[365–367] Attachment to the apical membrane, but without penetration into or through the PTC monolayer barrier, resulted in rapid and selective termination of blood flow to the adjacent area, and thus resulted in vascular isolation of the infected area with localized hypoxia, leukocyte migration, and necrosis. The same *E. coli* strain, missing only one virulence factor, required a far longer time to initiate this protective process. Tissue concentrations of cytokines were markedly elevated in the affected area compared with none in the injected areas.[367] Finally, prevention of this microvascular response resulted in widespread organ dissemination of the injected *E. coli* and death of the rat within 24 hours, something not seen with the intact system.[367] Therefore, communication between PTC and endothelial cells may lead to localization of the infecting agent and prevention of systemic spread.

Tamm-Horsfall protein (also known as *uromodulin*), a heavily glycosylated protein uniquely produced in the kidney by TALs,[368,369] modulates kidney innate immunity and inflammation during kidney injury.[362,368,370] Uromodulin knockout mice compared with WT controls subjected to kidney IRI showed increased S3 injury and necrosis,[370,371] neutrophil infiltration in the outer medulla, and expression of TLR4 and CXCL2 by S3 segments.[370,371] Neutralization of CXCL2 was protective, suggesting that a TLR4-CXCL2 proinflammatory pathway may be important in the pathophysiology and supporting uromodulin-dependent TAL-S3 crosstalk. Indeed, after IRI, a shift of trafficking of uromodulin was demonstrated toward the interstitium and basolateral aspects of S3 segments,[372] where a putative receptor for uromodulin is expressed.[370] This translocation of uromodulin was not the result of altered polarity of TAL. Uromodulin knockout ($THP^{-/-}$) mice, in addition to worse injury compared with WT mice, had an impaired transition of renal macrophages toward an M2 healing phenotype, suggesting that interstitial THP may not only regulate mononuclear phagocyte number, but plasticity and phagocytic activity as well.[373] A significant increase in uromodulin expression was shown in the kidney at the onset of recovery, which was concomitant with the suppression of tubular-derived cytokines and chemokines such as MCP-1, supporting the concept that the protective crosstalk mediated by uromodulin may be important in modulating recovery from AKI.[372]

COMPLEMENT

The complement system is part of the host defense machinery that protects against microbial invasion after injury.[374] The reactivity and specificity of the complement system is accomplished via a series of circulating pattern recognition proteins (PRPs) that sense PAMPs and initiate the complement cascade. The immunomodulatory functions of complement are mediated through three canonical pathways of activation—classic pathway, alternative pathway, and lectin pathway. The classic pathway is initiated by binding of PRPs to immune complexes, whereas the lectin pathway is initiated by binding PRPs to carbohydrate structures exposed in injured cells. The alternative pathway amplifies the initial response and maintains a low level of activity via a tick-over mechanism. On recognition of foreign surfaces, PRP-associated serine proteases cleave soluble components deposited on the activating surface and form C3 convertase complexes. Subsequent convertase-mediated cleavage of C3 leads to the formation of C3 fragments that serve as ligands for a variety of complement receptors that mediate increased phagocytosis and stimulation of the adaptive immune response. In addition to C3 convertase formation, opsonization also leads to the formation of C5 convertase, which activates C5 and initiates the formation of the pore-forming membrane attack complex (MAC) that lyses susceptible microorganisms or damages cells.[375]

Previous studies have suggested that complement is only located in the intravascular space, with most components synthesized in the liver. Many reports have now documented the presence of a functionally intact intracellular complement system within lymphocytes and epithelial, endothelial, and other cell types, and several studies have identified C3- and C5-mediated activation and signaling events in the intracellular space.[376]

Complement activation causes kidney injury through direct effects on renal cells and through interactions with cells of the innate and adaptive immune systems. Small soluble peptides, named "anaphylatoxins" (C3a and C5a) are generated during complement activation. These fragments trigger a systemic inflammatory response through their receptors, including vascular changes and chemotaxis of immune cells.[377] C3a and C5a receptors are expressed on leukocytes, endothelial cells,

mesangial cells, and tubular epithelial cells.[378] During ischemic injury, CR5a expression is markedly upregulated on proximal tubule epithelial cells, as well as interstitial macrophages. C5a is a powerful chemoattractant that recruits inflammatory cells. Complement cascades are activated during sepsis, and C5a, a potent complement component with procoagulant properties, is elevated in rodent models of sepsis. Blocking C5a or its receptor has shown some promise in improving survival with sepsis.[379]

To protect host cells from uncontrolled complement activation, several complement regulatory proteins are expressed in the surface of kidney cells and can directly inactivate complement convertase. Previous studies have documented complement activation in the pathogenesis of IRI-induced AKI.[380] These investigators used mice with a global deficiency to factor B or antibodies to factor B to demonstrate that the inhibition of C3 formation in kidney tissue protects mice from IRI-induced AKI. In addition to AKI, recent studies have demonstrated increased expression of complement C3 fragments and anaphylatoxin receptors C3aR and C5aR1 in PDGFRβ-positive pericytes and immune cells that were isolated from fibrotic kidneys. Inhibition of complement activation using mice with a global deletion of C3 has resulted in reduced tubulointerstitial fibrosis.[381] Given the increased awareness of the role of complement activation, not only with AKI but also in models of CKD, it is likely that emerging therapies aimed at inhibiting complement activation could be used in future studies to reduce the evolution of AKI and/or the progression of CKD.

INTRACELLULAR MECHANISMS

Reactive Oxygen Species

Oxidative stress plays an important role in the pathogenesis of AKI and progressive kidney disease. Low-level ROS can function as signaling molecules for cellular proliferation and vascular homeostasis under healthy conditions; however, increased ROS generation during IRI by mitochondria, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, or inflammatory cells can aggravate tissue injury. Therefore, reducing oxidative stress is considered an important therapeutic strategy to ameliorate loss of kidney function.[382]

ROS such as OH^-, peroxynitrite ($ONOO^-$), and hypochlorous acid (HOCl) are generated in epithelial cells during ischemic injury by catalytic conversion. These ROS can damage cells in a variety of ways (e.g., via peroxidation of lipids in plasma and intracellular membranes). They can also destabilize the cytoskeletal proteins and integrins required to maintain cell-cell adhesion, as well as extracellular matrix. These ROS can also have vasoconstrictive effects by their capacity to scavenge NO.[383]

Heat Shock Proteins

Much of the previous discussion has been on proteins or mechanisms that promote injury. However, there are protective mechanisms that allow cells to have a defense against numerous stresses. The complex heat shock protein (HSP) system is induced to exceptionally high levels during stress conditions. The HSPs are believed to facilitate the restoration of normal function by assisting in the refolding of denatured proteins, along with aiding the appropriate folding of newly synthesized proteins. They also help in the degradation of irreparable proteins and toxins to limit their accumulation. Overexpression of HSPs before injury has protective effects.[384–386] The proteins HSP90, HSP72, and HSP25 in particular have been extensively studied (e.g., overexpression of HSP25 is protective against actin cytoskeleton disruption).[387] After renal ischemia, cytosolic HSP90 is rapidly induced in PTCs, particularly in late stages, leading to the conclusion that HSP90 may be crucial for the disposition of damaged proteins and the assembly of newly formed peptides. Intrarenal transfection with HSP90 protects against IRI with the restoration of endothelial nitric oxide synthase (eNOS)–HSP90 coupling, eNOS activating phosphorylation, and Rho kinase levels, suggesting that HSPs can regulate the NO–eNOS pathway and intrarenal vascular tone.[388] In nephrotoxic models, HSP72 has been shown to limit apoptosis through an increased Bcl-2/Bax ratio, implicating HSP72 in cell death as well.[387]

Nayak's group has described the role of myoinositol oxygenase (MIOX), a proximal tubule enzyme that participates in oxidant injury by increasing the generation of ROS in kidney tissue.[389] In a more recent study,[390] the investigators used the cisplatin model of AKI in tubule-specific MIOX-overexpressing transgenic (MIOX-TG) mice and MIOX knockout ($MIOX^{-/-}$) mice. Compared with cisplatin-treated WT mice, cisplatin-treated MIOX-TG mice had more pronounced kidney injury, evidenced by higher serum concentrations of urea, creatinine, and kidney injury molecule 1 (KIM-1), along with more prominent apoptosis, but these effects were attenuated in cisplatin-treated $MIOX^{-/-}$ mice. $MIOX^{-/-}$ mice also had reduced NADPH oxidase-4 expression and ROS generation after cisplatin treatment. Increased inflammatory cells and upregulation of cytokines were found in kidneys of cisplatin-treated MIOX-TG mice. These findings suggest that MIOX overexpression leads to worsening of AKI in cisplatin-induced injury via multiple mechanisms and that MIOX gene disruption ameliorates toxic PTC injury.

To protect cells from harmful oxidative stress, the Keap1-Nrf2 system allows the cell to sense and respond to oxidative stress. The Keap1-Nrf2 pathway is a master regulator of cytoprotective genes and a promising target for therapeutic intervention. Nrf2 (nuclear factor–like 2) is a transcription factor that can bind antioxidant response elements (AREs) in target gene regulatory regions. In kidney IRI, Nrf2 signaling is upregulated in response to injury, and Nrf2 null mice developed worse disease.[391] It was also found that bardoxolone methyl ameliorates IRI through Nrf2.[392]

Given the known adverse effects of using pharmacologic enhancers of the Keap1/Nrf2 pathway,[393] a recent study used genetically altered mice that express low levels of Keap1 protein.[394] Reduction in levels of this inhibitor of Nrf2 signaling leads to enhanced Nrf2 target transcription. The investigators demonstrated a protective effect of Nrf2 enhancement in the model of ischemia-mediated AKI, as well as in the model of unilateral ureteral obstruction. These results underscore the importance of developing drugs that selectively target increased Nrf2 transcription to ameliorate AKI.

Iron, Ferritin, and Heme Oxygenase

Iron plays a central role in fundamental biologic functions, such as in mitochondrial respiration and DNA repair, yet at the same time plays a detrimental role in the pathophysiology of various diseases.[395] Circulating iron is mostly transferrin-bound.

A small amount bound to low-molecular-weight chelates, referred to as *labile* or *catalytic iron* is available in biologic systems and is thought to be responsible for kidney injury.[396] Although iron must undergo cyclic oxidation and reduction to perform its normal functions, this redox activity generates free radicals, leading to lipid peroxidation or generation of superoxide radicals through reaction with hydrogen peroxide (Haber-Weiss reaction).[397] The toxicity of catalytic iron to macromolecular components of the cell and its causal role in mediating disease have been demonstrated through the protection achieved by iron chelators.[62,396,398–402] Heme iron is derived primarily from erythrocyte turnover and is the major contributor to iron cycling in the body.[395]

Kidney tissue iron content increases after AKI,[403–406] suggesting that regulators of iron metabolism such as hemojuvelin and hepcidin could be used as therapeutic targets for the treatment of AKI. In a recent study, Scindia and coworkers reported that IRI caused an increase in serum iron and kidney nonheme iron levels.[404] They further demonstrated a significant reduction in IRI-induced apoptosis, oxidative stress, and inflammatory cell infiltration when hepcidin was administered 24 to 48 hours before IRI. Similar protection by the use of hepcidin was reported in the model of hemoglobin-mediated AKI.[407] Ferritin is the major regulator of intracellular iron and is composed of heavy-chain ferritin (H-ferritin) and light-chain ferritin (L-ferritin).[98] H-ferritin has ferroxidase activity converting Fe^{2+} to Fe^{3+}, which permits the incorporation of reactive iron into ferritin to avoid iron-induced ROS generation. Mice with proximal tubules deficient of H-ferritin had higher mortality, worse tissue injury and renal function, and increased apoptosis in both cisplatin and rhabdomyolysis models of AKI. Notably, there was disrupted iron trafficking, with altered distribution of ferroportin (iron export transporter) in the proximal tubule of H-ferritin–deficient mice. These results support an important role of proximal tubule H-ferritin and iron trafficking during AKI.[98]

Heme Oxygenase-1

The enzyme HO-1 has also emerged as a prominent player in renal tubular epithelial cell injury.[408–411] The biologic actions of HO-1 include antiinflammatory, vasodilatory, cytoprotective, and antiapoptotic effects and regulation of cellular proliferation in the setting of AKI. HO-1 is arguably one of the most readily inducible genes, responding to numerous stressors, including, but not limited to, hypoxia, hyperthermia, oxidative stress, and exposure to LPS. Induction of HO-1 has been described in various forms of AKI, including ischemic, endotoxic, and nephrotoxic models. Following ischemia-reperfusion, aged mice exhibit reduced induction of renal medullary HO-1 and worse renal injury than younger mice. A number of studies have indicated a protective effect of induction of HO-1 in AKI.[408–411] Prior induction of HO-1 by hemoglobin can reduce endotoxemia-induced renal dysfunction and mortality. Inhibition of HO-1 activity in the intact, disease-free kidney reduces medullary blood flow without exerting any effect on cortical blood flow. Overexpression of HO-1 by hemin results in a significant reduction in cisplatin-induced cytotoxicity,[412,413] and TNF-α–induced apoptosis in endothelial cells is also attenuated by the induction of HO-1.

These findings have been supported by studies of HO-1–deficient mice where glycerol-induced AKI resulted in marked exacerbation of renal insufficiency and mortality.[414] Mechanisms of HO-1–mediated protection have been extensively studied by Inguaggiato and coworkers, who showed that overexpression of HO-1 in cultured renal epithelial cells induces upregulation of the cell cycle inhibitory protein p21 and confers resistance to apoptosis.[415] Recently, HO-1 has been shown to attenuate ferroptosis in proximal tubular cells.[416] Induction of ferroptosis (erastin and RSL3) was less profound in $HO\text{-}1^{+/+}$ compared with $HO\text{-}1^{-/-}$ PTCs. Treatment with ferrostatin-1, a ferroptosis inhibitor, significantly improved viability. Macrophages in which HO-1 is upregulated by adenoviral strategies also protect against ischemic AKI. Fibroblasts from organs of transgenic pigs expressing HO-1 are resistant to proapoptotic stressors and exhibit a blunted proinflammatory response to LPS or TNF-α. Pretreatment with hemin augments glomerular HO-1 expression and renal expression of thrombomodulin and endothelial cell protein C receptor (EPCR) while reducing LPS-induced renal dysfunction, glomerular thrombotic microangiopathy, and the procoagulant state. Hemin also increases plasma levels of activated protein C in this model, suggesting its important role in the endothelial-epithelial axis in AKI.

Perhaps more importantly, HO-1 might also contribute to the repair and regeneration of tubular cells.[101,417] Following an acute insult, HO-1 is rapidly induced, but its expression subsides before renal recovery fully occurs; such abatement in HO-1 expression may allow the continued expression of proinflammatory and fibrogenic genes. In this regard, HO-1 deficiency promotes epithelial-mesenchymal transition, a process that may underlie the transition of AKI to CKD.[409] HO-1 gene expression is regulated differently in mice and humans; however, a recent study with a novel humanized transgenic mouse has confirmed the rescue of the pathologic phenotypes observed in $HO^{-/-}$ mice by the human HO-1 gene.[408] These mice offer an important tool to study the mechanisms of regulation of human HO-1 gene. In addition, the protective effects of HO-1 promoter polymorphisms in AKI could allow us to understand its significance better in clinical contexts and enable the identification of potential novel therapeutic targets.

REPAIR AND REGENERATION

The human kidney subjected to mild injury has the ability to recover renal function, as assessed by serum creatinine levels. Experimentally, the renal tubule has the capacity to undergo regeneration within a few days after AKI.[418] Early studies that focused on progenitor-stem cells in tubular epithelial cell injury found that different types of stem cells may reside in the renal architecture. In the human kidney, $CD133^{+}$ progenitor-stem cells with regenerative potential have been identified.[419–423] Chimeric studies using enhanced GFP to label donor bone marrow have revealed the lack of significant contribution of hematopoietic cells in the repair of epithelium following injury.[424]

Currently, there are likely two major hypotheses to explain the recovery of renal function by tubule regeneration[425] (Fig. 28.10). The first hypothesis suggests that tubules regenerate from any surviving tubular epithelial cells without the contribution of a preexistent intratubular stem cell or progenitor population. Genetic fate mapping studies have demonstrated that regeneration following injury is achieved primarily by surviving epithelial cells.[426,427] Lineage-tracing studies have demonstrated that fully differentiated epithelial cells that

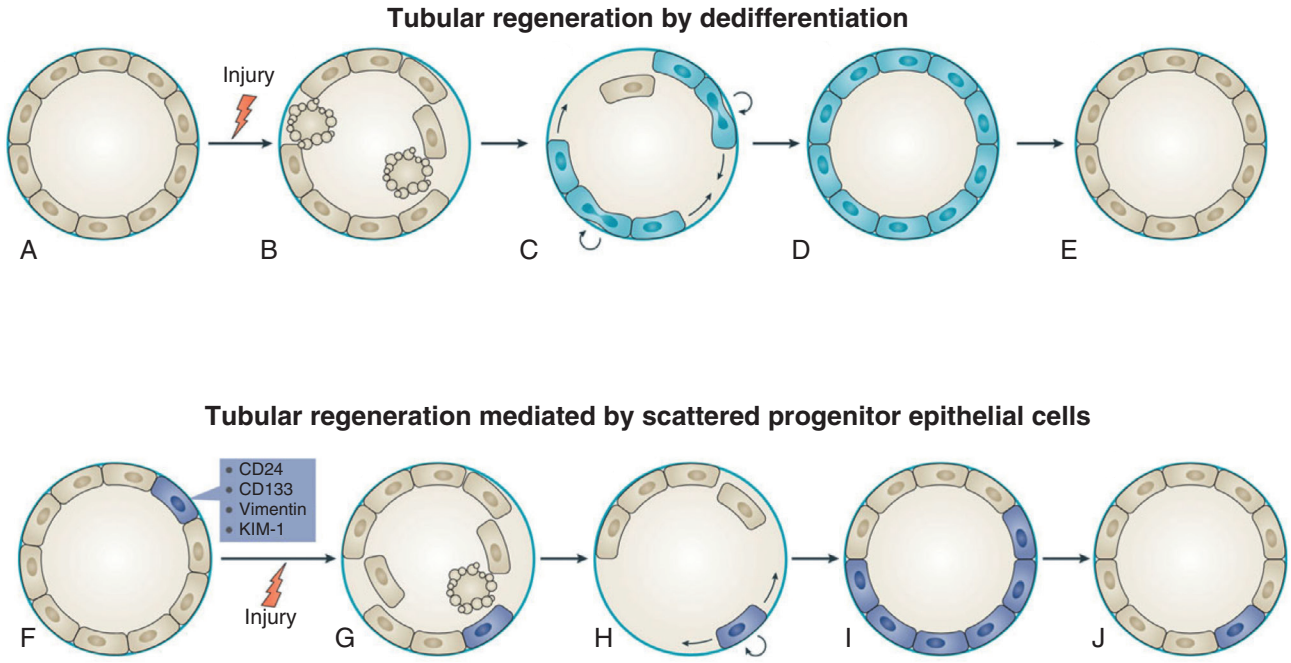

Fig. 28.10 Tubular regeneration following acute kidney injury. *Top panel,* Tubular regeneration by dedifferentiation. (A) Healthy tubules consist of nonproliferative mature epithelial cells that express markers of differentiation. (B) After injury, the epithelium is lost through apoptosis and necrosis. (C) Surviving epithelial cells dedifferentiate, either in response to sublethal injury signals or owing to signals from other injured cells, and acquire a proliferative phenotype. (D) The surviving dedifferentiated cells reconstitute the nephron epithelium. (E) Ultimately, most dedifferentiated cells redifferentiate and downregulate the expression of dedifferentiation genes. *Lower panel,* Tubular regeneration mediated by scattered progenitor epithelial cells. (F) Scattered tubular epithelial cells express signal transducer CD24, prominin-1 (CD133), and other genes that are characteristic of proximal tubule dedifferentiation, such as vimentin and KIM-1. (G) After injury, mature tubular cells, but not scattered cells, undergo apoptosis. (H, I) Scattered tubular cells expand in response to injury and their progeny reconstitute the tubule. (J) The small subpopulation of scattered tubule cells is preserved after regeneration. (From Chang-Panesso M, Humphreys BD. Cellular plasticity in kidney injury and repair. *Nat Rev Nephrol.* 2017;13(1):39–46.)

survive an acute kidney injury episode undergo a process of reversible dedifferentiation and proliferation during repair.[428] Another independent group used the same methodology of genetic cell mapping and doxycycline-inducible parietal epithelial cell mapping (PEC)–specific transgenic mouse to label proximal tubules in the model of IRI. The authors found a significant increase in genetic labeling of proximal tubules, with increased expression of scattered tubular cell biomarkers such as CD24 and CD133, during ischemia and subsequent recovery, suggesting that scattered tubular cells arise from any surviving tubular cell.[429]

The second hypothesis suggests that tubules regenerate from a specific tubular cell subpopulation with high regenerative potential or so-called *scattered tubular cells*[428,430–432] (see Fig. 28.10). A recent study supports this hypothesis and challenges the current paradigm that functional recovery after AKI relates to a regenerative capacity of all tubular epithelial cells. These investigators applied a lineage tracing approach using conditional Pax8-Confetti mice and the models of ischemia-reperfusion and glycerol-induced AKI to demonstrate the following: (1) AKI involves a permanent loss of tubular epithelial cells (TECs), even when GFR recovery occurs; (2) Pax2-positive TECs located in the S3 segment of the proximal tubule are endowed with a higher resistance to death and are responsible for the spontaneous regeneration of necrotic tubules after AKI; (3) only Pax2-positive cells complete mitosis while other TECs go through a process of endoreplication-mediated hypertrophy that accounts for the recovery of renal function; and (4) this process of endoreplication is the dominant cell response on AKI in mice and can be detected in renal biopsy tissue of patients who developed CKD after AKI.[433] The therapeutic targeting of tubule progenitors carrying specific biomarkers of scattered tubular cell phenotype could be a promising new strategy to improve long-term outcomes after AKI.

Growth factors and signals from injured cells are crucial at this stage to promote the timely and appropriate regenerative capacity of the viable cells. In animal models, administration of exogenous growth factors accelerates renal recovery from injury. These include epidermal growth factor, IGF-1, α-melanocyte stimulating hormone, erythropoietin, hepatocyte growth factor, and bone morphogenic protein-7 (BMP-7).[434–437] These effects have not yet been validated in human clinical trials of ATN.[438,439] They all likely increase the GFR through direct hemodynamic effects and may therefore hasten tubular epithelial cell recovery.

Extracellular vesicles (EVs) derived from bone marrow mesenchymal stromal cells promote the regeneration of kidneys in models of AKI.[440] EVs represent a heterogenous group of vesicles derived from nearly all mammalian cells. Smaller vesicles or exosomes range in size from 30 to 100 nm and larger microparticles from 100 to 1000 nm.[441] Microvesicles administered to severe combined immunodeficient (SCID) mice subjected to glycerol-induced AKI induced proliferation of tubule cells and accelerated morphologic and functional recovery.[442] Tubule proliferation was mediated by

RNA-dependent effects because RNase abolished the effects of microvesicles on this process.[442] When various populations of EVs were isolated by differential centrifugation, Bruno and coworkers found that transfer of 10 K (isolation at 10,000 g) and 100 K (isolation at 100,000 g) preparations into mice had different effects on AKI. The 100 K EVs contained mRNAs regulating proliferative, antiapoptotic, and growth factors, whereas the 10-K EVs had lower expression of proproliferative molecules and were unable to induce renal regeneration.[440]

Microvascular Function

The microvasculature consists of the endothelium and pericyte. Each of these structures contributes to barrier integrity for normal homeostatic function. During AKI, disruption may occur at each of these sites, leading to increased permeability and cell death. Normally, the microvasculature controls vascular tone, regulation of blood flow to local tissue beds, modulation of coagulation and inflammation, and permeability. Both ischemia and sepsis have profound effects on the endothelium. The renal vasculature and endothelium are particularly sensitive to these insults. When such an insult occurs, the endothelial bed becomes ineffective in performing its function, and the ensuing vascular dysregulation leads to continued ischemic conditions and further injury following the initial insult, which has been termed the "extension phase" of AKI.[443] Histopathologically, this is seen as vascular congestion, edema formation, diminished microvascular blood flow, and margination and adherence of inflammatory cells to endothelial cells.

Vascular Tone. Conger and associates were among the first to demonstrate that postischemic rat kidneys manifest vasoconstriction in response to decreased renal perfusion pressure and hence cannot autoregulate blood flow, even when total renal blood flow had returned to baseline values up to 1 week after injury.[444,445] Single-fiber, laser Doppler flow cytometry has revealed that blood flow is reduced to 60% and 16% of preischemic values in cortex and medulla, respectively and increased 125% in inner medulla following ischemia.[446] Selective inhibition, depletion, or deletion of iNOS has clearly shown renoprotective effects during ischemia.[447,448] NO production from the endothelium (eNOS) may be impaired at the level of enzyme activity or modified by ROS to impair normal vasodilatory activity.[449] In ischemic AKI,[450] there is an imbalance of eNOS and iNOS, and a relative decrease in eNOS, secondary to endothelial dysfunction and damage, leads to a loss of antithrombogenic properties of the endothelium and increased susceptibility to microvascular thrombosis.[450] Administration of L-arginine, the NO donor molsidomine, or the eNOS cofactor tetrahydrobiopterin can preserve medullary perfusion and attenuate AKI induced by IRI. Conversely the administration of N^{ω}-nitro-L-arginine methyl ester, an NO blocker, aggravates the course of AKI following IRI.[451,452]

In a review of experimental sepsis, the pattern of renal blood flow is inconsistent. Renal blood flow was decreased in 62% of studies and unchanged or increased in 38%.[453] Langenberg and colleagues administered *E. coli* to sheep and induced hyperdynamic sepsis associated with increased cardiac output and a decrease in mean arterial pressure and AKI, as evidenced by a rise in creatinine levels.[453] Furthermore, there was marked increase in renal blood flow, decrease in urine output, and decrease in creatinine clearance. When angiotensin was dose-titrated and restored blood pressure to presepsis levels, these effects were reversed; there was a significant decrease in renal blood flow, increase in urine output, and increase in creatinine clearance.[454] These studies suggest that improvement of function may be more than simply an improvement in mean arterial pressure and that the increase in glomerular filtration pressure may be through selective constriction of efferent arterioles and/or increase in mesangial cell tone.[454] Based on these principles, in a recent clinical trial, patients with vasodilatory shock who were receiving another vasopressor were randomized to Ang II or placebo. The group that received Ang II had higher mean blood pressure[455] and, in a subgroup of patients with AKI who required renal replacement therapy, 28-day survival, mean arterial pressure, and rate of discontinuation of renal replacement were higher in the angiotensin group. These results together show the potential benefit of Ang II in hyperdynamic sepsis, which may in part be due to improved glomerular hemodynamics.

The endothelial cell structure consists of a cytoskeletal structure of actin filament bundles that form a supportive ring around the periphery, along with the adhesion complexes that provide the integrity of the endothelial layer. The assembly and disassembly of actin filaments is regulated by a large family of actin-binding proteins, including ADF-cofilin. With ischemic injury, the normal architecture of the actin cytoskeleton is markedly changed, along with endothelial cell swelling, impaired cell-cell and cell-substrate adhesion, and loss of tight junction barrier functions. ATP depletion of cultured endothelial cells has been shown to induce dephosphorylation and activation of ADF-cofilin in a direct and concentration-dependent fashion. This activity results in depolymerized and severed actin filaments, seen as F-actin aggregates at the basolateral aspects of the cell.[456]

Kidney pericytes are extensively branched cells that are scattered on the outer wall of capillaries and embedded in the capillary basement membrane; they serve a homeostatic role in angiogenesis and vessel maturation.[457] Furthermore, following kidney injury, they detach and migrate into the kidney interstitium[458] and, in some cases, transform into myofibroblasts, leading to progressive kidney fibrosis. Thus, the endothelial-pericyte crosstalk among the vascular endothelial growth factor receptors and platelet-derived growth factor receptors contribute to normal microvascular health and disease. The importance of kidney pericyte microvascular function has been demonstrated through the use of diphtheria toxin delivery to FoxD1Cre::RsDTR transgenic mice in which the diphtheria toxin receptor (heparin-binding epidermal growth factor receptor) was selectively expressed in FoxD1 cells, which are primarily pericytes.[459] The administration of diphtheria toxin selectively ablated pericytes only. Within 96 hours, there was an abrupt decline in renal function and an increase in albuminuria. These results demonstrate that pericytes are essential for normal microvascular function.

The glycocalyx is located on the blood side of the endothelium and has a depth of 1 to 3 μm. The glycocalyx is composed of cell-bound proteoglycans, glycosaminoglycan side chains, and sialoproteins.[460] Sandwiched between blood components and the endothelium, the glycocalyx is situated to play a key role in microvascular and endothelial homeostasis

and endothelial physiology.[459] The specific functions served by the endothelial glycocalyx include shear stress mechanotransduction to endothelial cells, regulation of vascular permeability, inhibition of intravascular thrombosis, and protection of the endothelium from platelet and leukocyte adhesion.[460,461] The glycocalyx may be damaged by local changes in shear stress, ROS, altered oncotic pressure, altered fluid composition, and inflammatory molecules. Shedding of the glycocalyx occurs, exposing the endothelial adhesion molecules to circulating immune cells and leading to immune cell activation and inflammation and lipoprotein leakage to the subendothelial space. Coronary ischemia-reperfusion leads to shedding of the glycocalyx and an increase in neutrophil adhesion with subsequent myocardial injury.[462] Similar events are likely to occur in septic AKI and kidney IRI (Fig. 28.11).

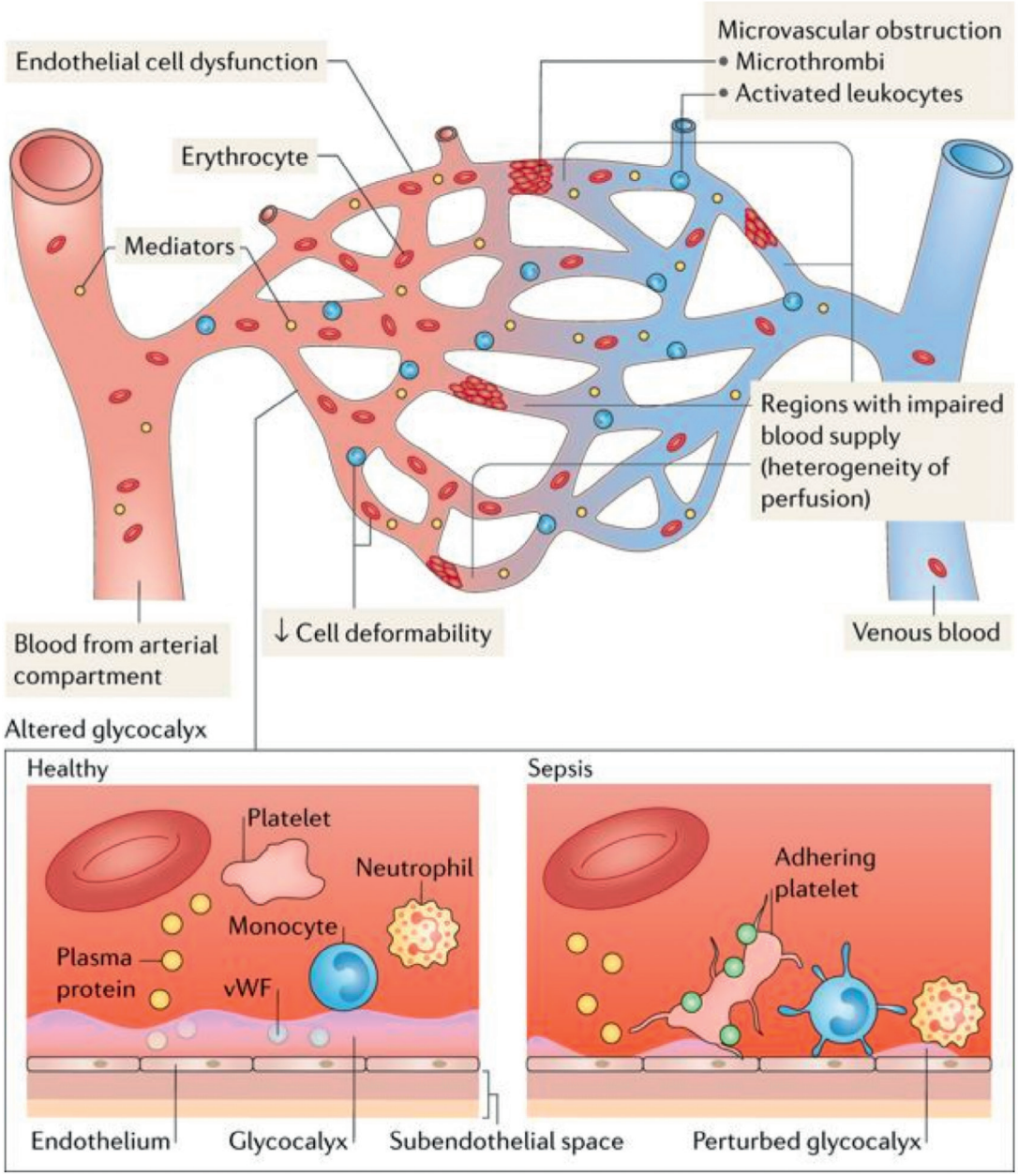

Fig. 28.11 Microvascular dysfunction in septic acute kidney injury. Multiple mechanisms are involved in the development of sepsis-related microvascular dysfunction, among which endothelial dysfunction (related partly to circulating host-derived and pathogen-derived mediators, as well as to reactive oxygen species) and an altered glycocalyx have major roles. The glycocalyx is a thin layer of glycosaminoglycans that covers the endothelial surface, facilitating the flow of red blood cells and limiting the adhesion of leukocytes and platelets to the endothelium. The glycocalyx may be substantially altered during sepsis, so interactions between the vascular endothelium and circulating cells (e.g., leukocytes and platelets) are impaired, and leukocyte rolling and adhesion to the endothelium may occur. Activation of coagulation and the generation of microthrombi might also participate in sepsis-induced microvascular alterations, as well as alterations in erythrocyte deformability and/or their adhesion to the endothelium. All these phenomena might cause heterogeneity in microvascular blood flow, with a decrease in vascular density and nonperfused capillaries, resulting in an increased diffusion distance for oxygen and in alterations in oxygen extraction. *vWF,* von Willebrand factor. (From Lelubre, C, Vincent, JL: Mechanisms and treatment of organ failure in sepsis. *Nat Rev Nephrol.* 2018;14:417–427.)

Within 24 hours after ischemic injury, there is loss of localization in vascular endothelial cadherin immunostaining, suggesting severe alterations in the integrity of the adherens junctions of the renal microvasculature.[463] In vivo two-photon imaging has demonstrated a loss of capillary barrier function within 2 hours of reperfusion, as evidenced by leakiness of high-molecular-weight dextrans (>300,000 Da) into the interstitial space.

Critical constituents of the perivascular matrix, including collagen IV, are known to be substrates of matrix metalloproteinase-2 (MMP-2) and MMP-9, which are collectively known as gelatinases. Breakdown of barrier function may also be due to MMP-2 or MMP-9 activation, and this upregulation is temporally correlated with an increase in microvascular permeability.[443,463] MMP-9 gene deletion stabilizes microvascular density following ischemic AKI, in part by preserving tissue VEGF levels.[464] In addition, minocycline, a broad-based MMP inhibitor and the gelatinase-specific inhibitor ABT-518, both ameliorated the increase in microvascular permeability in this model. Taken together, many studies have indicated that the loss of endothelial cells following ischemic injury is not a major contributor to altered microvascular permeability, although renal microvascular endothelial cells are vulnerable to the initiation of apoptotic mechanisms following ischemic injury, which can ultimately reduce microvascular density.[465]

The endothelial cell plays a central role in coagulation via its interaction with protein C through the EPCR and thrombomoduli. The protein C pathway helps maintain normal homeostasis and limits inflammatory responses. Protein C is activated by thrombin-mediated cleavage, and the rate of this reaction is further augmented (by 1000-fold) when thrombin binds to the endothelial cell surface receptor protein thrombomodulin. The activation rate of protein C is further increased by approximately 10-fold when EPCR binds protein C and presents it to the thrombin-thrombomodulin complex. Essentially, activated protein C then has antithrombotic actions and profibrinolytic properties and participates in numerous antiinflammatory and cytoprotective pathways to restore normal homeostasis.[466] Based on these properties, the endothelial cell plays an absolutely essential and critical role in maintaining a normal and healthy vasculature and endothelial bed.

In AKI, microvascular function is ultimately compromised, resulting in disseminated intravascular coagulation and microvascular thrombosis, decreased tissue perfusion, and hypoxemia, leading to organ dysfunction and failure. It has been shown that both pretreatment and postinjury treatment with soluble thrombomodulin attenuates renal injury, with minimization of vascular permeability defects and improvement in capillary renal blood flow.[467]

The leukocyte and endothelial cell are dynamically involved in the process of adherence of leukocytes to the vascular endothelium. Leukocyte activation and cytokine release require signals through chemokines circulating in the bloodstream or through direct contact with the endothelium. Rolling leukocytes can be activated by chemoattractants such as complement C5a and platelet-activating factor. Once activated, leukocyte integrins bind to endothelial ligands to promote firm adhesion. β_2-Integrin (CD18) seems to be the most important ligand for neutrophil adherence. These interactions with the endothelium are mediated through endothelial adhesion molecules that are upregulated during ischemic conditions.[468]

The initial phase starts with slow neutrophil migration mediated by tethering interactions between adhesion molecules and their endothelial cell ligands. Within 2 to 4 hours of IRI or sepsis, endothelial P-selectin and intercellular adhesion molecule 1 (ICAM1) are expressed on endothelial cells associated with leukocyte trapping in peritubular capillaries.[306] Singbartl and Ley have found that platelet P-selectin and not endothelial P-selectin is the main determinant in neutrophil-mediated ischemic kidney injury.[468] There is also significant protection from both ischemic injury and mortality by blockade of the shared ligand to all three selectins (E-, P-, and L-selectin), which seems to be dependent on the presence of a key fucosyl sugar on the selectin ligand.[469] In a CLP model of septic azotemia, mice deficient for E-selectin, P-selectin, or both were completely protected. Furthermore, selectin-deficient mice demonstrated similar intraperitoneal leukocyte recruitment but altered cytokine levels when compared with WT mice,[470] in addition to engagement in leukocyte-endothelial cell interactions.[470] Other adhesion molecules such as VAP-1 appear to play a role in AKI.[315]

Recently, in an attempt to transition to human studies, Dehnadi and coworkers have demonstrated the critical role that leukocyte integrin CD11b–CD18 contribute to both AKI and CKD in nonhuman primates.[471] In their model, using a CD11b–CD18 inhibitor, mAb107, ischemia-reperfusion-induced a no-reflow time; plasma creatinine and ATN were markedly diminished in the mAB107 groups, indicating that the leukocyte integrin CD11b–C18 contributes to AKI. Moreover, subsequent assessment up to 9 months after AKI revealed improvement in microvascular perfusion and histology.

Recent studies have examined the role of microparticles (MPs) in sepsis-related AKI. MPs are cell membrane-derived particles of 0.2 to 2 μM in diameter that can promote coagulation and inflammation.[472] Using the cecal ligation model of sepsis, the authors found that calpastatin overexpression improved survival, organ dysfunction (including lung, kidney, and liver damage), and lymphocyte apoptosis. Increased calpastatin expression also decreased the sepsis-induced systemic proinflammatory response and disseminated intravascular coagulation by reducing the number of procoagulant circulating microparticles and therefore delaying thrombin generation.[473] The deleterious effect of microparticles in this model was confirmed by transferring microparticles from septic WT to septic transgenic mice, worsening their survival and coagulopathy. The development of therapeutics aimed to prevent the release of MPs from endothelium or other inflammatory cells may be a reasonable target in microvascular dysfunction during sepsis-induced AKI.

ACUTE KIDNEY INJURY TO CHRONIC KIDNEY DISEASE TRANSITION

AKI is one of many initiating events that contribute to chronic, progressive kidney disease.[474,475] Some patients with AKI fully recover kidney function, whereas in others, the development of CKD is accompanied by a progressive decline in kidney function, leading ultimately to end-stage kidney disease (ESKD).[476–478] Regardless of the cause of CKD (e.g., nephrotoxic kidney injury, ischemia, infection, genetics; paraneoplastic, immunologic processes), there is a stereotypical response leading to interstitial fibrosis, tubular atrophy, and

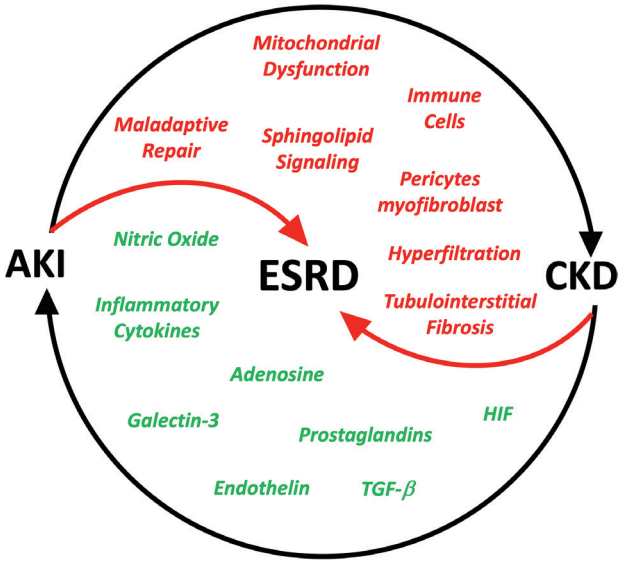

Fig. 28.12 Conceptual framework of acute kidney injury *(AKI)* to chronic kidney disease *(CKD)* transition. AKI can lead to CKD and then to end-stage renal disease (ESRD). Conversely, CKD can lead progressively to ESKD or can predispose to AKI, which then enters a vicious circle and predisposes to CKD. A number of factors contribute to AKI to CKD transition, including traditional concepts *(green)* and emerging *(red)* concepts.

peritubular rarefaction and inflammation.[474,479,480] Incomplete repair following AKI contributes to the progression to CKD and ESKD[418,481] (Fig. 28.12).

Role of the Endothelium

Studies have begun to elucidate the importance of the endothelium in the initiation and progression of fibrosis. Studies on human biopsies have associated microvascular rarefaction with progressive kidney failure.[482] There is also evidence in experimental AKI that acute injury to endothelial cells may have long-term implications; Basile have shown a significant reduction in blood vessel density following ischemic injury, leading to the phenomenon of vascular dropout.[483] Vascular dropout was verified by Horbelt and associates, who found a drop in the vascular density by almost 45% at 4 weeks after an ischemic insult.[465] Ehling and coworkers, using micro-CT imaging of vascular alterations, found a progressive decline of the renal relative blood volume in three models of progressive kidney disease (IRI, unilateral ureteral obstruction [UUO], and Alport) of up to 61% in late disease stages and that it preceded fibrosis.[484] Using ex vivo micro-CT imaging and three-dimensional quantification of the renal vasculature, they demonstrated macrovascular to microvascular alterations in progressive renal disease, as evidenced by a reduction in vessel diameter and in vessel branching and increased vessel tortuosity (Fig. 28.13). These findings suggest that unlike the renal epithelial tubular cells, the renal vascular system lacks comparable regenerative potential. Recent studies have suggested that renal endothelial injury can result in capillary rarefaction due to hypoxia and a lack of upregulation of VEGF in response to injury.[485–487] It is not yet clear whether apoptosis and/or necrosis play a major role in endothelial cell dropout. Ischemia has been shown to inhibit the angiogenic protein VEGF while inducing ADAMTS1, which is thought to be a VEGF inhibitor.[488] It was then postulated that the lack of vascular repair could be due to lack of VEGF, as shown by experiments where administration of VEGF-121 preserved the microvascular density.[488] Reduction of the microvasculature density increases hypoxia-mediated fibrosis and alters usual hemodynamics, which may lead to hypertension. Thus, loss of microvasculature density and its consequential effects may play a critical role in the progression of CKD following initial recovery from ischemia-reperfusion–induced AKI.[465,483,484] Using a genetic approach in the IRI mouse model of AKI, the temporal effects of endothelial sphingosine 1-phosphate receptor 1 (S1P1) during AKI were examined. Delayed deletion of S1P1 in endothelial cells after IRI, using a tamoxifen-inducible system to delete S1P1 prevented kidney recovery, resulting in chronic inflammation and progressive fibrosis. Specifically, S1P1 directly suppressed the endothelial activation of leukocyte adhesion molecule expression and inflammation. Altogether, the data indicate that activation of endothelial S1P1 is necessary to protect from IRI and permit recovery from AKI.[359]

Epigenetic Modification

AKI is associated with histone modification[489] and DNA methylation,[490] leading to altered transcription of genes thought to contribute to renal injury.[491,492] There is evidence that epigenetic changes are closely related to renal hypoxia and CKD progression.[485,486]

Renal Oxidative Stress in Acute Kidney Injury–Chronic Kidney Disease Transition

Renal oxidative stress has been shown to persist with resultant increased Ang II sensitivity in rats following recovery from ischemia-reperfusion–induced AKI after 5 weeks. Post-AKI rats showed significantly enhanced renal vasoconstrictor responses to Ang II, and treatment of AKI rats with apocynin normalized these responses. Expression of mRNA for common reduced NADPH oxidase subunits was not altered in kidneys following recovery from AKI; however, mRNA screening using polymerase chain reaction assays has suggested that post-AKI rats have decreased renal glutathione peroxidase 3 mRNA and an increased expression of other pro-oxidant genes such as lactoperoxidase, myeloperoxidase, and dual oxidase-1. Following infusion with Ang II, renal fibrosis was enhanced in post-AKI rats. The profibrotic response was significantly attenuated in rats treated with apocynin. These data suggest that there is sustained renal oxidant stress following recovery from AKI that alters the hemodynamic and fibrotic responses to Ang II and may contribute to the transition to CKD following AKI.[493] This also highlights the importance of managing recovering AKI very carefully to avoid further insults that might enhance ROS or sensitivity to Ang II.

Cell Cycle Arrest

As discussed earlier, in successful repair, mature surviving cells dedifferentiate, proliferate, and repopulate proximal tubules in response to kidney injury.[426,427] Upregulation of KIM-1 is antiinflammatory through its engulfment and clearance of dead and apoptotic cells[494] but becomes maladaptive when chronically expressed.[495] Repetitive injury may result in the upregulation of cyclin-dependent kinase (CDK) inhibitors, $p16^{Ink4}$ and $P53\text{-}p21^{Cip1/Waf1}$, leading to cell cycle arrest.[496]

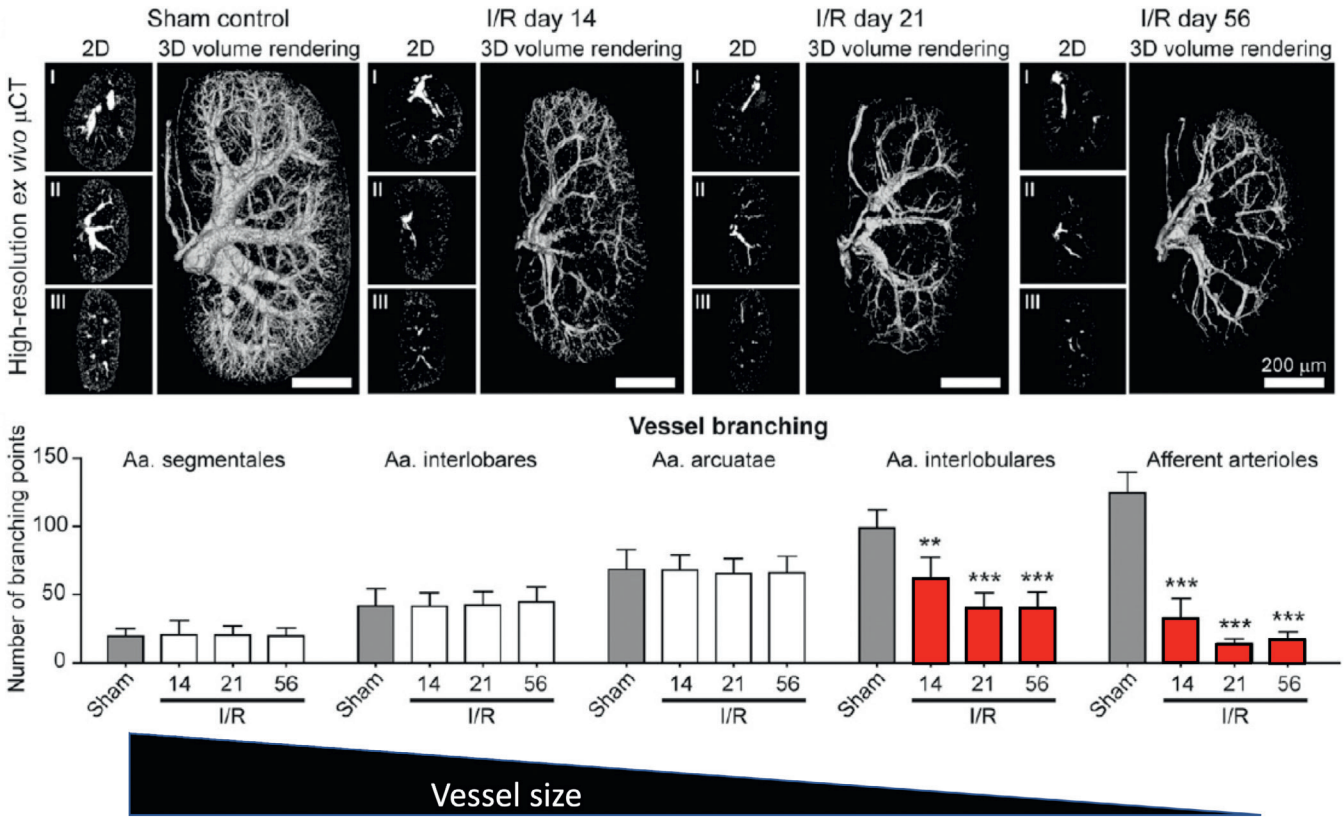

Fig. 28.13 Ex vivo micro-computed tomography *(CT)* imaging and three-dimensional quantification of the renal vasculature reveal significant alterations of renal arteries in progressive ischemia-reperfusion (IRI) induced renal injury. *(Lower panel)* Representative high-resolution, ex vivo, micro-CT images of sham control and I/R kidneys from days 14, 21, and 56 after Microfil perfusion (two-dimensional cross-sectional images in transverse [I], coronal [II], and sagittal [III] planes, as well as three-dimensional volume renderings). Note the progressive rarefaction of functional vessels besides the continuous shrinkage of fibrotic kidneys over time. Scale bar, 200 µm. *(Upper panel)* Micro-CT–based quantification of vascular branching points per increasing vessel order (from hilus to periphery). A continuous reduction in vessel branching and vessel size was linked to an increased vessel tortuosity during fibrosis progression. **, $P < .01$; ***, $P < .001$. *AKI,* Acute kidney injury. (From Ehling J, Babickova J, Gremse F, et al. Quantitative Micro-computed tomography imaging of vascular dysfunction in progressive kidney diseases. *J Am Soc Nephrol.* 2016;27(2):520–532.)

An increase in $p16^{Ink4}$ inhibits CDK4 and CDK6 and is associated with tubulointerstitial changes leading to fibrosis; kidneys from $INK4a^{-/-}$ mice develop less fibrosis following kidney IRI.[250] Thus, cell cycle arrest and cellular senescence lead to resistance to apoptosis and persistent metabolic activity, promoting a senescence-type secretory phenotype with the secretion of TGF-β, cytokines, proteases, and other proinflammatory and profibrotic factors.[418,497] Yang, Bonventre and associates have used four models of kidney injury, including severe bilateral IRI, unilateral IRI, aristolochic acid, and UUO and demonstrated that G2/M arrest contributes to maladaptive repair and fibrosis associated with AKI-CKD transition. Similar findings have been reported and, importantly, pharmacologic inhibitors of G2/M arrest reduce fibrosis.[498,499]

Inflammation in Acute Kidney Injury–Chronic Kidney Disease Transition

During kidney recovery, renal and extrarenal cells participate in the wound-healing response and can initiate fibrosis. Immune cells of the mononuclear phagocyte system, including macrophages and dendritic cells, not only contribute to injury but have emerged as important cells in the recovery of kidney function during adaptive repair or fibrosis during maladaptive repair. It is the balance between wound healing and progressive fibrosis that dictates the final outcome. The intrinsic plasticity of monocytes-macrophages and dendritic cells, as well as attempts to relate in vitro studies to in vivo findings, makes the functional definition and phenotype of this myeloid population in kidney pathophysiology complex.[500–503] In vitro studies have led to two well-defined mononuclear phagocytes. Classically activated macrophages—M1 mononuclear phagocytes consisting of macrophages and dendritic cells—are produced by exposure to LPS or INF-γ and are largely thought to be proinflammatory and contribute to initial kidney injury. Alternatively, activated macrophages (M2 mononuclear phagocytes) are induced by IL-4 and IL-10 and appear later, after AKI has been established, and have a genetic signature associated with wound healing and/or fibrosis.[504] These mononuclear phagocyte phenotypes depend on the complex local tissue microenvironment, which may induce phenotype switching.[303]

A key feature of fibrosis is the activation of extracellular matrix–producing myofibroblasts.[505,506] Other factors relevant in CKD progression include endothelial cell damage and vascular damage in AKI,[483] hypoxia-inducible factor (HIF),[507] innate and adaptive immunity,[508] cell cycle arrest,[509] and epigenetic mechanisms.[510,511]

Although some injured tubules undergo repair and regeneration, injury may also be accompanied by inflammation, maturation and proliferation of fibroblasts, and extracellular matrix deposition as part of the process of fibrosis. The source of fibroblasts in the injured kidney—fibrocytes, epithelial cells through epithelial mesenchymal transformation (EMT), intrinsic fibroblasts, and pericytes—remains controversial.[512,513]

Tissue fibrosis is a common component of inflammatory diseases, including various forms of CKD. Collagen deposition, characteristically formed during normal wound healing, is reversible but progressive irreversible fibrosis may occur through repeated injury due to dysregulated inflammation.[514] Some studies have focused on the role of pericytes or resident fibroblasts that contribute to normal homeostatic microvascular function but transform into myofibroblasts on activation.[513,515,516] Myofibroblasts are the primary collagen-producing cells.[512]

Although the myofibroblast is the cell type responsible for depositing collagen, investigators have been searching for its precursors. Other than interstitial fibroblasts that are known to transition, pericytes, dendritic, endothelial, and epithelial cells have been identified as potential contributors. For example, in vivo and in vitro studies have demonstrated that endothelial cells develop a myofibroblast phenotype.[517,518] Epithelial cells grown in culture can produce genes expressed in myofibroblasts, suggesting epithelial to mesenchymal transition.[519–521] Fate-mapping studies by Humphreys and associates have shown that although epithelial cells can obtain mesenchymal markers in vitro, they do not penetrate the basement membrane to enter into the interstitial space and differentiate into myofibroblasts in vivo.[513] Furthermore, lineage analysis has demonstrated that platelet-derived growth factor receptor (PDGFR)-β–positive pericytes differentiate into myofibroblasts in a UUO model. Following AKI, a potential step in the AKI-CKD transition is the dissociation of pericytes from endothelial cells.[458] Using bigenic Gli1-CreERt2; R26tdTomato reporter mice, Kramann and coworkers observed dissociation between $Gli1^+$ pericytes and endothelial cells after AKI.[458] Furthermore, following pericyte ablation, endothelial damage was observed, and pericyte loss led to a significantly reduced capillary number, a hallmark of CKD.

Two studies have shed light on this controversy. *Snai1* (encodes snail family zinc finger 1, Snail1) expressed in mouse renal epithelial cells is required for kidney fibrosis.[522] Twist (encodes twist family bHLH transcription factor 1, known as Twist) genes are essential regulators of EMT.[523] Inhibition or deletion of these genes or overexpression has led to induction or inhibition of EMT, respectively.[524,525] Lineage tracing studies have demonstrated that during activation, labeled epithelial cells do not contribute to myofibroblasts or interstitial cells; however, there was downregulation of epithelial markers and loss of epithelial polarity and cell differentiation.[525] Thus, partial EMT leads to cell cycle arrest, blocks proliferation and repair, and blocks secretion of growth factors, such as TGF-β that drive proliferation of myofibroblasts[526] (Fig. 28.14).

Signaling pathways Wnt, hedgehog (Hh), and Notch pathways also play important roles during renal fibrosis. In CKD, numerous Wnt ligands are upregulated, resulting in prolonged activation of the Wnt/β-catenin pathway.[527,528] Epithelial β-catenin activation leads to epithelial dedifferentiation and interstitial fibrosis.[529] Wnt derived from tubules is necessary for myofibroblast activation and interstitial fibrosis.[530] Recently, the role of the sphingolipid pathway in progressive fibrosis. Sphingosine 1-phosphate (S1P), a pleiotropic lysophospholipid that is involved in diverse functions such as cell growth and survival, lymphocyte trafficking, and vascular stability,[531–536] is the product of sphingosine phosphorylation by two sphingosine kinase isoforms (SphK1 and SphK2). SphK2 has been localized to the nucleus and plays an important role in the production of nuclear S1P, which acts as a histone deacetylase (HDAC) inhibitor,[537] thereby allowing gene expression to be induced. Bawja demonstrated that following unilateral IRI or folic acid (FA)–induced kidney fibrosis, protection of $Sphk2^{-/-}$ mice from fibrosis at day 14 was thought to be due to IFN-γ. Kidneys of $Sphk2^{-/-}$ mice exhibited greater expression of *Ifng* and IFN-γ–responsive genes (Cxcl9 and Cxcl10) than kidneys of WT or $Sphk1^{-/-}$ mice. IFN-γ blocking antibody administered to $Sphk2^{-/-}$ mice or deletion of *Ifng* using a double-knockout mouse ($Sphk2^{-/-}$ $Ifng^{-/-}$ mice) blocked the protective effect of *Sphk2* deficiency in fibrosis. Moreover, adoptive transfer of $Sphk2^{-/-}$ CD4 T cells into WT mice blocked FA-induced fibrosis. Finally, a selective SphK2 inhibitor blocked FA-induced kidney fibrosis in WT mice. These studies have demonstrated that SphK2 inhibition may serve as a novel therapeutic approach for attenuating kidney fibrosis.[538,539]

Activation of myofibroblasts is also mediated through paracrine signals from immune cells (lymphocytes and antigen-presenting cells), which include cytokines, chemokines, angiogenic factors, growth factors, peroxisome proliferator-activated receptors (PPARs), and other molecules.[540] Although Th2 cytokines may induce fibrosis, Th1 cytokines such as IL-12 or IFN-γ have been shown to inhibit pulmonary[541,542] and experimental renal fibrosis.[543]

Using an unbiased approach to identify upregulated and downregulated pericyte genes in injury, Schrimpf and colleagues found increased activation and expression of ADAMTS1 (a disintegrin-like metalloprotease with thrombospondin type 1) and downregulation of its inhibitor MMP-3 (TIMP-3).[544] TIMP-3–stabilized pericytes maintained collagen capillary tube networks, whereas ADAMTS1-treated pericytes led to enhanced destabilization. TIMP-3 has many functions, including regulating VEGF signaling, pericyte migration, and MMP-2 and MMP-9 activity.[545] Furthermore, $TGF\text{-}\beta_1$ has been shown to activate the pericyte-myofibroblast transition, thus adding to the stimuli for pericyte involvement in fibrosis.[546] Injured epithelial cells reduce production of VEGF, a trophic factor for endothelial maintenance, and increase production of TGF-β and PDGFR, which enhance pericyte dedifferentiation into myofibroblasts. Finally, PDGFR blockade on pericytes or VEGF receptor 2 (VEGFR-2) on endothelial cells led to a reduction in fibrosis and stabilization of the microvasculature in the UUO model.[547] Thus, numerous factors favor microvascular maladaptation after injury, including TGF-β production and lack of VEGF production by epithelial cells, reductions in PDGF production by endothelial cells, and increased ADAMTS1 expression, with reduced TIMP-3 production by pericytes. These events also suggest several therapeutic targets that could limit microvascular dropout and loss of kidney function secondary to the fibrotic process. Normal pericyte function therefore becomes deranged following transformation to

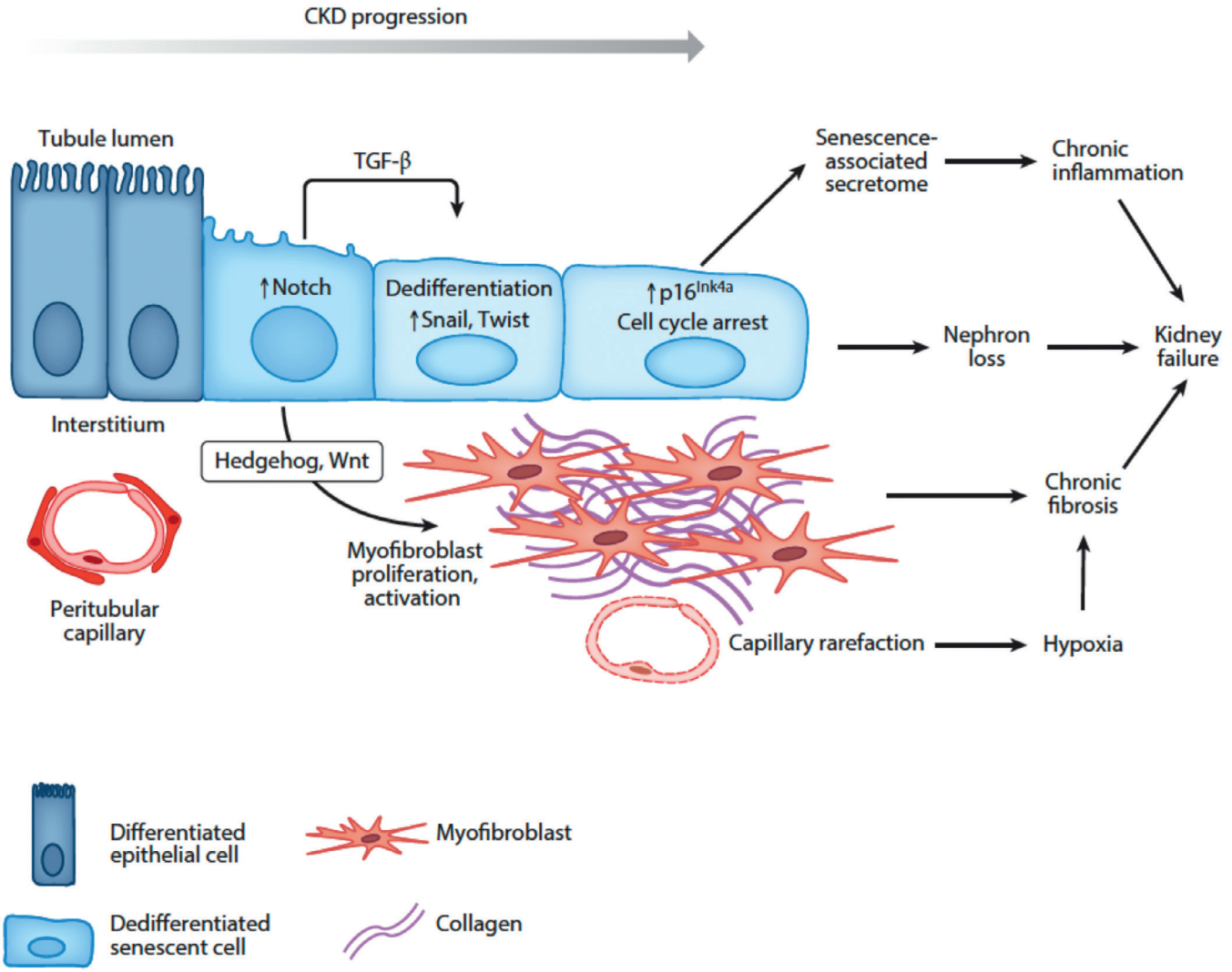

Fig. 28.14 Critical cells and signaling pathways activated in progressive chronic kidney disease *(CKD)*. CKD is initiated by cellular injury, either to the epithelial or endothelial compartment. In the tubulointerstitium, injury leads to epithelial dedifferentiation that may be induced by transforming growth factor-β (TGF-β) and Notch pathway upregulation. Dedifferentiated epithelial cells secrete paracrine signaling factors, such as hedgehog and Wnt ligands, that act on interstitial pericytes and mesenchymal stem cell–like cells to activate myofibroblast differentiation, proliferation, and matrix secretion. This in turn causes peritubular capillary rarefaction and ongoing hypoxia. Chronic tubular injury leads to epithelial cell cycle arrest and senescence, with accompanying secretion of proinflammatory cytokines that amplify inflammation. These interrelated events ultimately drive nephron loss, ongoing interstitial fibrosis, and kidney failure. (From Humphreys BD. Mechanisms of renal fibrosis. *Annu Rev Physiol.* 2018;80:309–326.)

activated myofibroblasts, which leads to loss of microvascular stability, ineffective angiogenesis, increased permeability, and capillary rarefaction, which are all processes leading to fibrosis.[457,474]

Proximal tubules are highly metabolically active, relying on aerobic metabolism. Mitochondrial fatty acid oxidation is a major source of energy, and mitochondrial dysfunction leads to lipid accumulation, ATP depletion, and fibrosis in proximal tubule cells.[548] A number of therapeutic approaches are in development, including some agents in clinical trials for diabetic nephropathy and other kidney diseases,[549,550] but as yet there are no approved treatments to prevent the development of kidney fibrosis or accelerate repair. The development of effective treatments requires a better understanding of the inflammatory, injury, wound healing, matrix deposition, and cellular repair processes that accompany fibrosis.[497,514,526,551–553] For example, mitochondria may be an important therapeutic target in AKI to CKD progression.[554] In one approach, Birk and associates developed a novel mitoprotective drug, SS31.[555] SS peptides bind selectively to cardiolipin in the mitochondrial inner membrane to protect mitochondrial cristae. SS31 allows better function of the respiratory chain complexes, prevents peroxidase activity, and enhances electron transfer through cytochrome c to improve oxidative phosphorylation and ATP production.[555–557] SS31 administered 1 month after renal IRI attenuated glomerulosclerosis and senescence and reduced parietal epithelial cell activation and changes in podocyte and endothelial structure.[558] Perry and associates have examined the role of dynamin-related protein 1 (DRP1), a mediator of cell fission.

Proximal tubule-specific deletion of *Drp1* prevented renal IRI, inflammation, and programmed cell death and promoted epithelial recovery, which was associated with activation of the renoprotective β-hydroxybutyrate signaling pathway.[559] Importantly, delayed deletion of proximal tubule *Drp1* using a tamoxifen-inducible system after IRI attenuated kidney fibrosis. These results highlight DRP1 and mitochondrial dynamics as an important mediator of AKI and progression to fibrosis and suggest that DRP1 may serve as a therapeutic target for AKI.

Effects of Organ Crosstalk

AKI is associated with significant morbidity and mortality. In the intensive care unit, where patients with AKI sustain multiorgan dysfunction, the mortality rate is around 60%.[560] Although AKI is associated with consequences such as accumulation of uremic toxins, metabolic acidosis, fluid and electrolyte imbalance, and fluid overload, these factors alone do not explain the mortality associated with AKI.[146,561] There is accumulating evidence that in many cases, AKI is a systemic condition in which AKI induces dysfunction in distant organs, including lung, heart, brain, liver, and intestines (Fig. 28.15). Moreover, there is evidence that distant organ function can alter kidney function. Recent studies have demonstrated that neural control of inflammation and AKI is thought to be mediated by a pathway referred to as the *cholinergic antiinflammatory pathway*, which requires an intact spleen.[562–566]

Kelly has demonstrated the effects of renal ischemia on cardiac tissues.[139] Induction of IL-1, TNF-α, and ICAM-1 mRNA was seen in cardiac tissues as early as 6 hours after renal ischemic injury and remained elevated up to 48 hours after renal ischemic injury. There was also a significant increase in myeloperoxidase activity in the heart and liver, apart from the kidneys. The increase in cardiac myeloperoxidase activity could be prevented by the administration of anti–ICAM-1 antibody at the time of renal ischemia. At 48 hours, cardiac function evaluation by echocardiography also revealed increases in left ventricular end-systolic and diastolic diameter and decreased fractional shortening. As little as 15 minutes of ischemia also resulted in significantly more apoptosis in cardiac tissue.[139] In transgenic sickle mice, bilateral renal IRI results in marked cardiac vascular congestion and increased serum amyloid P component (the murine equivalent of C-reactive protein). Kramer and colleagues have shown that renal ischemic injury leads to an increase in pulmonary vascular permeability defects that are mediated through macrophages.[135] Furthermore, they noted that in a rat model of bilateral renal ischemic injury or nephrectomy, there was downregulation of the lung epithelial Na channel, Na^+-K^+-ATPase, and aquaporin-5 expression, but not in unilateral ischemic models, suggesting the role of uremic toxins in modulating these effects in the lung.[567] Liu and colleagues have also shown effects of AKI on functional changes in the brain.[568] Mice with AKI had increased neuronal pyknosis and microgliosis in the brain; there were increased levels of the proinflammatory chemokines keratinocyte-derived chemoattractant and granulocyte colony-stimulating factor in the cerebral cortex and hippocampus and increased expression of glial fibrillary acidic protein in astrocytes in the cortex and corpus callosum. In addition, extravasation of Evans blue dye into the brain suggested that the blood-brain barrier was disrupted in mice with AKI.

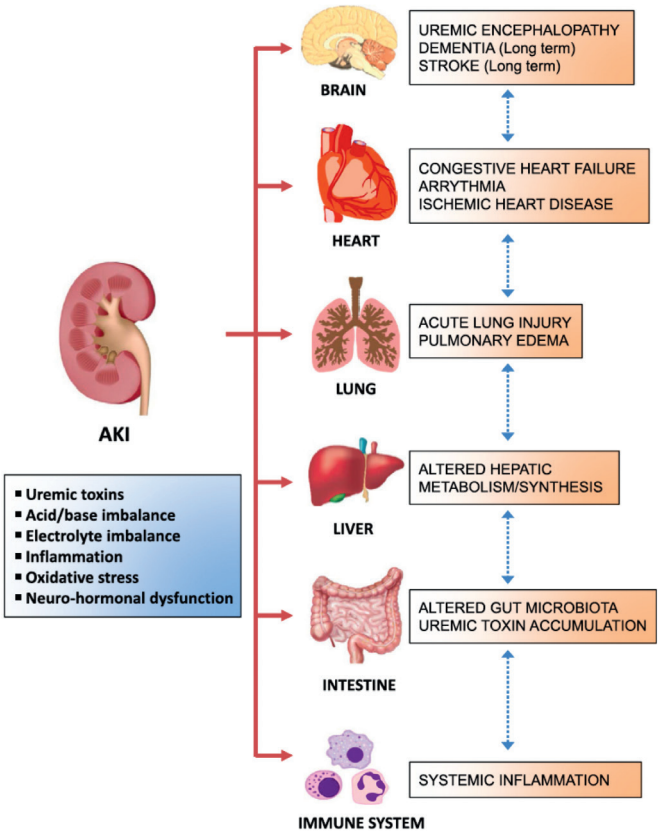

Fig. 28.15 The impact of acute kidney injury *(AKI)* on distant organs. AKI causes hemodynamic, humoral, and immunologic changes, which lead to dysfunction of distant organs, including lung, heart, brain, liver, and intestine and the immune system. (From Lee SA, Cozzi M, Bush EL, Rabb H. Distant organ dysfunction in acute kidney injury: a review. *Am J Kidney Dis.* 2018;72(6):846–856.)

Many of the same processes involved in kidney-lung, kidney-heart, and kidney-brain interactions have been observed in the liver—increased neutrophil infiltration, vascular congestion, and vascular permeability after AKI. Following experimental AKI, levels of hepatic TNF-α, IL-6, IL-17A, ICM-1, keratinocyte-derived chemoattractant, IL-10, and MCP-1 are increased. The presence of AKI before a second-hit ischemic hepatic injury exacerbates liver injury as well, suggesting ongoing crosstalk between the kidney and liver[146] (Table 28.1).

Other organs also regulate ischemic renal injury. Patients with acute lung injury frequently have AKI, in part due to mechanical ventilation. There are three potential mechanisms whereby mechanical ventilation induces AKI—the effects of changes in arterial blood gases, barotrauma-induced systemic release of inflammatory agents, and the influence on systemic and renal blood flow.[569] Imai and coworkers have demonstrated the role of lung injury in inducing renal damage.[570] They found that in rabbits, injurious lung ventilatory strategies (high tidal volume and low peak end-expiratory pressure) alone were sufficient to induce renal epithelial cell apoptosis. This finding was further substantiated by the fact that plasma obtained from rabbits that underwent the injurious ventilation strategy induced more pronounced apoptosis in cultured $LLC\text{-}RK_1$ cells in vitro, suggesting that circulating soluble

factors associated with mechanical ventilation might be involved in this process.

An example of extrarenal organs regulating ischemic AKI is evidenced by the effect of brain death on kidney transplants. Traumatic brain injury elicits a cytokine and inflammatory response. These cytokines result in renal inflammation in kidney transplants from brain-dead donors, distinct from living or cardiac-death donors.[571] Pretransplantation biopsies of brain-dead donor kidneys contain more infiltrating T lymphocytes and macrophages. Reperfusion of kidneys from brain-dead donors is associated with the instantaneous release of inflammatory cytokines, such as granulocyte colony-stimulating factor, IL-6, IL-9, IL-16, and MCP-1. In contrast, kidneys from living and cardiac death donors show a more modest cytokine response, with the release of IL-6 and small amounts of MCP-1.[572]

An important example of organ crosstalk is the one that occurs between the nervous and immune systems. Rather than two independent systems, these two systems are linked to maintain normal homeostasis and to respond to stress and pathophysiologic disorders. Studies have identified neural pathways that regulate immunity and inflammation via the inflammatory reflex pathway, thus identifying specific molecular targets that can be modulated by stimulating neurons electrically,[562,573,574] by ultrasound,[566,583] or optically.[564] The cholinergic antiinflammatory pathway (CAP) is the efferent limb of the so-called inflammatory reflex pathway mediated through the vagus nerve.[575–577] The afferent limb is activated by bacterial products (PAMPs), DAMPs, proinflammatory cytokines, immunoglobulins, and ATP.[578,579] Following detection of these inflammatory molecules at receptors, afferent signals from injured tissue and the immune cells are transmitted to the brain,[577,580] which then activates the vagus efferent nerve. Then the inflammatory reflex controls peripheral cytokine levels and inflammation through key cellular components such as macrophages and $CD4^+$ T cells. Indeed, vagus nerve stimulation shows antiinflammatory and organ-protective effects in various disorders, such as arthritis,[581] colitis,[582] ileus,[582] AKI,[563] and others. The spleen plays an integral role in linking the nervous and immune systems via the CAP. Prior splenectomy worsens AKI,[583] blocks the antiinflammatory effect of the CAP,[563,564] and worsens lung inflammation following kidney IRI.[584] Because AKI is associated with high mortality and morbidity, these studies indicate that multiorgan crosstalk that occurs in the setting of AKI is likely to be a major contributor to nonrenal organ dysfunction and may mediate clinically observable events, such as cardiac, pulmonary, and central nervous system events.

Finally, we address the issue of unilateral AKI affecting contralateral kidney function. In a model where the ischemia-reperfusion–injured kidney was removed after 5 weeks to isolate effects on the untouched kidney, challenge with higher dietary sodium yielded significant increase in blood pressure relative to sham-operated controls. Similarly, contralateral kidneys had impaired pressure natriuresis and hemodynamic responses, but reductions in vascular density were observed in the contralateral kidney. However, contralateral kidneys contained interstitial cells, some of which were identified as activated (low $CD62L/CD4^+$) T lymphocytes. Taken together, these data suggest that the salt-sensitive features of AKI on hypertension and CKD can be segregated so that effects on hemodynamics and hypertension may develop independently of direct renal damage.[585]

Complete reference list available at ExpertConsult.com.

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BOARD REVIEW QUESTIONS

1. Which of the following are true statements regarding the pathogenesis of acute kidney injury?
   a. Following acute tubule necrosis, remaining renal epithelial cells are thought to undergo cell division and repopulate the tubule epithelium.
   b. Following AKI, scattered progenitors cells are thought to repopulate tubule epithelium.
   c. During early phase of acute tubular necrosis, epithelial cell polarity is lost.
   d. None of the above
   e. All of the above

   Answer: e

   Rationale: In AKI there is loss of epithelial polarity due to the disruption of the actin cytoskeleton and the loss of tight junctions and adherens junctions. These junctional complexes actively participate in numerous functions, including paracellular transport, cell polarity, and cellular shape. Early ischemic injury results in "opening" of these tight junctions, leading to increased paracellular permeability causing further back leak of the glomerular filtrate into the interstitium. Following the acute insult, repair process are initiated. There are 2 major hypotheses to explain recovery of renal function by tubule regeneration. The first hypothesis suggests that tubules regenerate from any surviving tubular epithelial cells. Genetic fate mapping studies demonstrated that regeneration following injury was achieved primarily by surviving epithelial cells. The second hypothesis suggests that tubules regenerate from a specific tubular cell subpopulation with high regenerative potential or so called "scattered tubular cells".

2. Which of the following statements are true regarding the pathogenesis of acute kidney injury?
   a. Renal medulla is the most susceptible region for injury in ischemia-reperfusion injury.
   b. The oxygen tension (PO_2) is lowest in the medulla.
   c. Following ischemia-reperfusion injury, RBC flow decreases to the greatest degree in the medulla.
   d. None of the above
   e. All of the above

   Answer: e

   Rationale: The renal medulla is particular susceptible to AKI due to the low oxygen tension, a condition which is further exacerbated by endothelial cell activation facilitating leukocyte adherence as a consequence of expression of endothelial cell adhesion molecules. There is a decrease in RBC flow, leading to medullary circulatory congestion and reduced oxygen and nutrient supply to the outer medulla.

3. Which of the following are mechanisms of hypoxia in the kidney of chronic kidney disease?
   a. Loss of peritubular capillaries
   b. stagnation of peritubular capillary blood flow
   c. decreased oxygen delivery as a result of anemia.
   d. All of the above
   e. None of the above

   Answer: d

   Rationale: Recent studies have begun to elucidate the importance of the endothelium in the initiation and progression of fibrosis. Studies on human biopsies have associated microvascular rarefaction with progressive renal failure. Renal endothelial injury can result in a reduction of capillary density, called capillary rarefaction, occurring several weeks after AKI which may be due to hypoxia and a lack of upregulation of VEGF in response to injury. Capillary rarefaction may in turn affect tubular epithelial cells, (myo)fibroblasts, and inflammatory cells, leading to tubulointerstitial fibrosis. There is evidence that epigenetic changes are closely related to renal hypoxia and CKD progression. Lastly the anemia associated with CKD, in part, due to decreased erythropoietin production leads to a decrease in oxygen delivery.

4. Which of the following contributes to fibrosis in AKI to CKD transition?
   a. Persistent inflammation
   b. Pericyte transformation into myofibroblasts
   c. Direct proximal tubule damage
   d. All of the above
   e. None of the above

   Answer: d

   Rationale: A key feature of progression of AKI to CKD is the activation of extracellular matrix-producing myofibroblasts. Other factors important in CKD progression include proximal tubule cell, endothelial cell and vascular cell damage in AKI, increase in tubular oxygen consumption, increased oxidative stress that can affect the expression of Hypoxia-Inducible factors in renal cells. In addition, innate and adaptive immunity, cell cycle arrest and epigenetic mechanisms also contribute. While some injured tubules may undergo repair and regeneration, injury may also be accompanied by persistent inflammation, maturation and proliferation of fibroblasts, and extracellular matrix deposition as part of the process of fibrosis. The source of fibroblasts in the injured kidney - fibrocytes, epithelial cells through EMT, intrinsic fibroblasts and pericytes - remains controversial. Recent studies also suggest that in the absence of fibrosis persistent regulated epithelial cell necrosis may represent a pathophysiological mechanism of transition from AKI to CKD.

29

Prevention and Management of Acute Kidney Injury

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CHAPTER OUTLINE

DEFINITION OF ACUTE KIDNEY INJURY

Acute kidney injury (AKI) is a heterogeneous syndrome defined by rapid (hours to days) decline in the glomerular filtration rate (GFR), resulting in the retention of metabolic waste products, including urea and creatinine, and dysregulation of fluid, electrolyte, and acid-base homeostasis.[1] Although often considered a discrete syndrome, AKI represents a broad constellation of pathophysiologic processes of varied severity and cause. These include decreases in the GFR as the result of hemodynamic perturbations that disrupt normal renal perfusion without causing parenchymal injury, partial or complete obstruction to urinary flow, and a range of processes with characteristic patterns of glomerular, interstitial, tubular, or vascular parenchymal injury. AKI occurs in a heterogeneous patient population—genetics, age, kidney functional status, accompanying comorbidities—and the cause is often multifactorial.

The term *acute kidney injury* has largely supplanted the older terminology of acute renal failure (ARF). This change reflects recognition of serious shortcomings with the older terminology. Acute renal failure suggested a dichotomous relationship between normal kidney function and overt organ failure; in contrast, AKI captures the growing body of data associating small to moderate acute and transient decrements in kidney function with serious, adverse outcomes. Although the AKI terminology does emphasize the graded aspect of acute kidney disease, it should be recognized that this terminology is also imperfect. The term *injury* can be construed to imply the presence of parenchymal organ damage, which may be absent in a variety of settings associated with an acute decline in kidney function, such as early obstructive disease and prerenal azotemia associated with volume depletion. Although the term *acute kidney dysfunction* might better characterize the entire spectrum of the syndrome, AKI is the term that has been adopted by consensus and is now increasingly used in the medical literature.[2,3] In this chapter, AKI will be used to describe the entire spectrum of the syndrome. Although, in clinical practice, the term *acute tubular necrosis* (ATN) is often used synonymously with AKI, these terms should not be used interchangeably. Although ATN is the most common form of intrinsic AKI, particularly in critically ill patients, it represents only one of multiple forms of AKI. In addition, there may be a lack of concordance between the clinical syndrome and the classic histopathologic findings of ATN.[4,5]

Decreased urine output is a cardinal (although not universal) manifestation of AKI, and patients are often classified based on urine flow rates as nonoliguric (urine output >400 mL/day), oliguric (urine output <400 mL/day), or anuric (urine output <100 mL/day).[6] Transient oliguria may occur in the absence of significant decrements in kidney function, because increased tubular salt and water reabsorption is a normal physiologic response to volume depletion. In contradistinction, persistent oliguria, despite the presence of adequate intravascular volume, is virtually always a manifestation of AKI, with lower urine volume typically associated with more severe initial renal injury. The categorization of AKI based on urine volume has clinical implications for the development of volume overload, severity of electrolyte disturbances, and overall prognosis. Although oliguric AKI

is associated with a higher mortality risk than nonoliguric AKI, therapeutic interventions to augment urine output (see later) have not been shown to improve patient outcomes.[7]

AKI can develop de novo in the setting of intact kidney function or can be superimposed on underlying chronic kidney disease (acute on chronic kidney injury), and the presence of underlying impaired kidney function has been shown to be one of the most important risk factors for the development of AKI.[8,9] Multiple mechanisms may contribute to this increased susceptibility, including diminished renal functional reserve, impaired salt and water conservation predisposing to intravascular volume contraction, decreased activity of detoxification mechanisms, increasing susceptibility to cytotoxic injury, impaired clearance of potential nephrotoxins, increasing the risk for and/or duration of exposure, and associated macrovascular and microvascular disease, increasing the risk of ischemic injury.

The causes of AKI are usually divided into three broad pathophysiologic categories:

1. Prerenal AKI—diseases characterized by effective hypoperfusion of the kidneys in which there is no parenchymal damage to the kidney (Box 29.1);
2. Intrinsic AKI—diseases involving the renal parenchyma (Table 29.1); and
3. Postrenal (obstructive) AKI—diseases associated with acute obstruction of the urinary tract (Box 29.2)

Box 29.1 Causes of Prerenal Acute Kidney Injury

Intravascular Volume Depletion
- Hemorrhage—trauma, surgery, postpartum, gastrointestinal
- Gastrointestinal losses—diarrhea, vomiting, nasogastric loss
- Renal losses—diuretics, osmotic diuresis, diabetes insipidus,
- Skin and mucous membrane losses—burns, hyperthermia
- Nephrotic syndrome
- Cirrhosis
- Capillary leak

Reduced Cardiac Output
- Cardiogenic shock
- Pericardial diseases—restrictive, constrictive, tamponade
- Congestive heart failure
- Valvular diseases
- Pulmonary diseases—pulmonary hypertension, pulmonary embolism
- Sepsis

Systemic Vasodilation
- Sepsis
- Cirrhosis
- Anaphylaxis
- Medications

Renal Vasoconstriction
- Early sepsis
- Hepatorenal syndrome
- Acute hypercalcemia
- Drugs—norepinephrine, vasopressin, nonsteroidal antiinflammatory drugs (NSAIDs), angiotension-converting enzyme
- Calcineurin inhibitors

Iodinated Contrast Agents

Increased Intraabdominal Pressure

Abdominal Compartment Syndrome

Although these categories are useful for didactic purposes and help inform the initial clinical assessment of patients presenting with AKI, there is often a degree of overlap between these categories. For example, renal hypoperfusion may cause a spectrum of renal injury ranging from prerenal azotemia to overt ATN, depending on its severity and duration. As a result, precise categorization of the cause of AKI in these three groups may not always be possible, and individual patients may transition between categories regarding causation.

The prior absence of a uniform operational definition of AKI impeded epidemiologic studies and hampered clinical evaluations of preventive and therapeutic interventions. Older literature was characterized by multiple definitions using varying absolute and/or relative changes in the serum creatinine concentration, with or without associated decrements in urine output, which made it difficult to compare findings

Table 29.1 Major Causes of Intrinsic Acute Kidney Injury

Type of Injury	Examples
Tubular injury	
• Ischemia due to hypoperfusion	Hypovolemia, sepsis, hemorrhage, cirrhosis, CHF (see Box 29.1)
• Endogenous toxins	Myoglobin, hemoglobin, paraproteinemia, uric acid (see Table 29.3)
• Exogenous toxins	Antibiotics, chemotherapy agents, radiocontrast, phosphate preps. (see Table 29.3)
Tubulointerstitial injury	
• Acute allergic interstitial nephritis	Nonsteroidal antiinflammatory drugs (NSAIDs), antibiotics
• Infections	Viral, bacterial, and fungal infections
• Infiltration	Lymphoma, leukemia, sarcoid
• Allograft rejection	
Glomerular injury	
• Inflammation	Anti-GBM disease, ANCA-associated GN, postinfectious, cryoglobulinemia, membranoproliferative glomerulonephritis, IgA nephropathy, SLE, Henoch-Schonlein purpura, polyarteritis nodosa
• Hematologic	Hemolytic-uremic syndrome, thrombotic thrombocytopenic purpura, medications
Renal microvasculature	Malignant hypertension, toxemia of pregnancy, hypercalcemia, radiocontrast, scleroderma, medications
Large vessels	
• Arterial	Thrombosis, vasculitis, dissection, thromboembolism, atheroembolism, trauma
• Venous	Thrombosis, compression, trauma

ANCA, Antineutrophil cytoplasmic antibody; *CHF,* congestive heart failure; *GBM,* glomerular basement membrane; *GN,* glomerulonephritis; *IgA,* immunoglobulin A; *SLE,* systemic lupus erythematosus.

Box 29.2 Causes of Postrenal Acute Kidney Injury

Upper Urinary Tract Extrinsic Causes

Retroperitoneal space—lymph nodes, tumors
Pelvic or intraabdominal tumors—cervix, uterus, ovary, prostate
Fibrosis—radiation, drugs, inflammatory
Ureteral ligation or surgical trauma
Granulomatous diseases
Hematoma

Lower Urinary Tract Causes

Prostate—benign prostatic hypertrophy, carcinoma, infection
Bladder—neck obstruction, calculi, carcinoma, infection (schistosomiasis)
Functional—neurogenic bladder secondary to spinal cord injury, diabetes, multiple sclerosis, stroke, pharmacologic side effects of drugs (anticholinergics, antidepressants)
Urethral—posterior urethral valves, strictures, trauma, infections, tuberculosis, tumors

Upper Urinary Tract: Intrinsic Causes

Nephrolithiasis
Strictures
Edema
Debris, blood clots, sloughed papillae, fungal ball
Malignancy

across studies. In 2002, the Acute Dialysis Quality Improvement Initiative (ADQI) proposed the first consensus definition of AKI. The ADQI work group proposed a classification scheme with three strata based on the magnitude of increases in serum creatinine levels and/or the duration of oliguria (Table 29.2).

As proposed, the first stratum provides the greatest sensitivity for diagnosing AKI, whereas higher strata provide increasing specificity of diagnosis. These three strata were combined with two outcome stages defined by the need for and duration of renal replacement therapy, resulting in the five-tiered RIFLE classification (***r***isk of renal dysfunction, ***i***njury to the kidney, ***f***ailure of kidney function, as well as the two outcome stages, ***l***oss of kidney function and ***e***nd-stage kidney disease).[10] Subsequently, the Acute Kidney Injury Network (AKIN) modified the RIFLE classification by adding an absolute increase in serum creatinine level of 0.3 mg/dL or more to the 50% relative increase in the serum creatinine level to the definition of AKI and specifying that these increments develop over no more than 48 hours[2] (see Table 29.2). This definition was further modified in the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline for acute kidney injury, which clarifies that although the 0.3-mg/dL increment in the serum creatinine level needs to occur over 48 hours, from a known baseline value, the 50% increase may occur over a longer, 7-day interval.[3]

The KDIGO clinical practice guideline for AKI recognized a gap in the nosology of acute and chronic kidney disease.[3] Based on the definitions previously, AKI has an onset of less

Table 29.2 RIFLE, Acute Kidney Injury Network (AKIN) and Kidney Disease: Improving Global Outcomes (KDIGO) Definitions and Staging of Acute Kidney Injury

Definitions

Parameter	RIFLE	AKIN	KDIGO
Serum creatinine	An increase of >50% developing over <7 days	An increase of >0.3 mg/dL or of >50% developing over <48 hours	An increase of >0.3 mg/dL developing over <48 hours or an increase of >50% developing over <7 days
Urine output[a]	<0.5 mL/kg/h for >6 hours	<0.5 mL/kg/h for >6 hours	<0.5 mL/kg/h for >6 hours

Staging Criteria

Increase in Serum Creatinine

RIFLE		AKIN	KDIGO	Urine Output[a]
Risk	≥50%	Stage 1: ≥0.3 mg/dL or ≥50%	Stage 1: ≥0.3 mg/dL or ≥50%	<0.5 mL/kg/h for >6 hours
Injury	≥100%	Stage 2: ≥100%	Stage 2: ≥100%	<0.5 mL/kg/h for >12 hours
Failure	≥200%	Stage 3: ≥200%	Stage 3: ≥200%	<0.3 mL/kg/h for >24 hours or anuria for >12 hours
Loss	Need for renal replacement therapy for >4 weeks			
End-stage	Need for renal replacement therapy for >3 months			

[a]The urine output criteria for both definition and staging of AKI are the same for the RIFLE, AKIN, and KDIGO criteria.
RIFLE, ***R***isk of renal dysfunction, ***i***njury to the kidney, ***f***ailure of kidney function, as well as the two outcome stages, ***l***oss of kidney function and ***e***nd-stage kidney disease.

than 7 days, whereas chronic kidney disease (CKD) is defined by the presence of impaired kidney function or structural kidney damage for more than 3 months with implications for health.[11] Recognizing that some patients develop kidney disease with a more subacute onset than that of AKI, but of less than 3 months' duration, the KDIGO Acute Kidney Injury Workgroup proposed the concept of acute kidney disease (AKD), defined as AKI or a reduction in the GFR to less than 60 mL/min/1.73 m,[2] a decrease in the GFR by 35% or more, an increase in the serum creatinine level by more than 50%, or the presence of structural kidney damage of less than 3 months' duration.

Several limitations to these criteria for diagnosis and staging of AKI have been recognized.[12,13] First, although validation studies have demonstrated that the AKI stage correlates with mortality risk, it is not clear that this is the appropriate metric for assessing their validity as a definition of kidney disease. Second, there is poor correlation between AKI stage and the GFR. Because the magnitude of change in the serum creatinine level is time-dependent, a patient may demonstrate progression over time from less severe (RIFLE-R, AKIN, or KDIGO stage 1) to more severe AKI stage (RIFLE-F, AKIN, or KDIGO stage 3), despite an improving GFR. Third, the definition of AKI by serum creatinine criteria relies on a referent baseline serum creatinine level, which is often unavailable. Furthermore, variations in specifications for this referent value (e.g., admission serum creatinine level vs. most recent outpatient serum creatinine level prior to admission vs. other definitions) can alter the classification of patients.[14] Fourth, both RIFLE and AKIN definitions use relative changes in serum creatinine level to stage AKI. Analyses of creatinine kinetics have demonstrated that the time required to attain a fixed percentage change in serum creatinine level in the setting of severe AKI is dependent on the baseline level of kidney function, whereas the initial rate of change in serum creatinine level is relatively independent of kidney function.[12] Thus, early in the course of AKI, absolute changes in the serum creatinine level may be detected more readily than relative changes. Fifth, concordance between the serum creatinine level and urine output criterion is poor, even with regard to mortality risk.[15] Transient changes in urine output may reflect variation in volume status or be due to the administration of medications and do not necessarily correlate with other parameters of kidney function. Finally, it must be noted that these classification systems are independent of the various causes of AKI—prerenal, intrinsic, obstructive. Despite these shortcomings, the use of standardized classification schemes has enhanced the interpretation of epidemiologic studies and the design of clinical trials.

Conceptually, AKI comprises a spectrum of structural and functional kidney disease in which there may be an evolution from injury to organ dysfunction and, finally, overt organ failure. Reliance solely on changes in serum creatinine level and/or urine output to diagnose AKI has resulted in the inability to identify the incipient stages of intrinsic kidney damage, which may be the most opportune time for pharmacologic intervention.[16] To facilitate the early diagnosis of intrinsic injury, multiple biomarkers of tubular injury have been evaluated, including N-acetyl-β-D-glucosaminidase (NAG), kidney injury molecule 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), interleukin 18 (IL-18), liver fatty acid binding protein (L-FABP), tissue inhibitor of metalloproteinase 2 (TIMP-2), and insulin-like growth factor-binding protein 7 (IGFBP7).[17–22] In addition, serum cystatin C has been proposed as more sensitive (and, in some settings, more specific) than serum creatinine for detecting changes in the GFR, and urinary cystatin C has been proposed as a marker of tubular injury.[17,23,24] Although most of these biomarkers have yet to be adequately validated for routine clinical use, they have the potential to provide an earlier diagnosis of intrinsic AKI than the serum creatinine level, differentiate volume-responsive (prerenal) AKI from intrinsic disease, diminish the confounding effect related to creatinine generation, and provide prognostic information regarding the clinical course of an episode of AKI. One or more of these biomarkers may provide a means whereby patients could be identified at the incipient stage of AKI to guide the implementation of specific therapy to ameliorate kidney damage or promote recovery of kidney function.

INCIDENCE OF ACUTE KIDNEY INJURY

Estimates of the incidence of AKI are highly dependent on the case definition used, with rates among hospitalized patients ranging from as high as 44% when defined based on a change in serum creatinine level of at least 0.3 mg/dL to as low as 1% using an increase in serum creatinine level of at least 2.0 mg/dL.[25–29] Approximately 3% to 7% of hospitalized patients and 25% to 60% of intensive care unit (ICU) patients develop AKI, with 5% to 6% of the ICU population requiring renal replacement therapy after developing AKI.[25–29] In a single-center analysis conducted in 1996 at an urban tertiary care hospital, AKI, defined as an increase in serum creatinine level of 0.5 mg/dL for patients with a baseline serum creatinine level of 1.9 mg/dL or less, of 1.0 mg/dL for patients with a baseline serum creatinine level of 2.0 to 4.9 mg/dL, and 1.5 mg/dL for patients with a baseline serum creatinine level greater than 5 mg/dL, developed in 7.2% of 4622 consecutive patients.[25] The overall incidence of AKI is approximately 21.6% for all hospitalized adults worldwide,[30] with known associations for accelerating CKD to end-stage kidney disease (ESKD).[31–33] The more recent estimate of AKI incidence is considerably higher than the 4.9% investigators had observed in a similar study in 1979.[34] In the late 1970s, the most frequent cause for AKI was decreased renal perfusion, observed in 39% of episodes, followed by medication-associated (16%), contrast-associated (11%), postoperative (9%), and sepsis-associated (6.5%). Overall mortality was 19.4%, with higher mortality rates associated with larger maximal increments in the serum creatinine concentration.

Although definition is less of an issue with regard to rates of AKI requiring renal replacement therapy, reported rates vary considerably because of differences in the characteristics of patient populations and variability in criteria for the initiation of renal replacement therapy. In a multinational, multicenter observational study of 29,269 critically ill patients, 5.7% developed severe AKI and 4.3% received renal replacement therapy (RRT).[35] In a smaller but more recent multinational observational study, 13.5% of 1802 critically ill patients developed RRT requiring AKI.[29]

Many epidemiologic studies of AKI have relied on data from large administrative databases. Such data need to be

interpreted with caution, however, because administrative coding for AKI is incomplete and may only capture 20% to 30% of all episodes of AKI.[36,37] The ascertainment of AKI requiring RRT using administrative data is substantially more complete. In an analysis of data from the National Hospital Discharge Survey in the United States, the Centers for Disease Control and Prevention have observed an increase in hospital discharges with a diagnosis of AKI from 18/100,000 population in 1980 to 365/100,000 in 2005.[38] Similar trends have been observed in analyses of the US Nationwide Inpatient Sample (NIS) and a 5% sample of US hospitalized Medicare beneficiaries. In an analysis that combined administrative and clinical data from a single integrated health care delivery system, the incidence of AKI that did not require the use of RRT increased from 322.7 to 522.4/100,000 person-years from 1996 to 2003.[39] Over the same period, AKI requiring RRT increased from 19.5 to 29.5/100,000 person-years.[39] AKI was more common among men and older adults. In a more recent analysis using data from the NIS, a nationally representative administrative database of hospitalizations, the incidence of dialysis-requiring AKI increased from 222/million person-years in 2000 to 533/million person-years in 2009, with the largest rise in incidence occurring in patients 65 to 74 years of age and 75 years of age or older.[40] Although a component of these temporal trends may be attributable to earlier initiation of dialysis and more frequent use of RRT in older patients, these changes are unlikely to account for most of the increase in incidence of severe AKI.

Preexisting kidney disease is one of the major risk factors for the development of dialysis-requiring AKI.[41] More severe baseline CKD is associated with higher levels of risk. Compared with patients with a baseline estimated GFR (eGFR) more than 60 mL/min/1.73 m^2, patients with eGFR values of 45 to 59 mL/min/1.73 m^2 have a nearly twofold increased risk of developing dialysis-requiring AKI. This risk increases to more than 40-fold among patients with baseline eGFR values less than 15 mL/min/1.73 m^2.[41] Underlying diabetes mellitus, hypertension, and the presence of proteinuria are also associated with the risk for hospital-acquired AKI.

CATEGORIZATION OF ACUTE KIDNEY INJURY

Although in the clinical setting AKI is often multifactorial, the cause is commonly evaluated based on three major pathophysiologic categories—prerenal, intrinsic, and postrenal (obstructive) AKI. Prerenal azotemia is the most common cause of AKI, accounting for about 40% to 55% of all cases.[25,27,42] Prerenal AKI is caused by a reduction in blood flow to the kidneys that can result from absolute and/or effective reduction in intravascular volume. Common causes of absolute intravascular volume depletion leading to prerenal AKI include gastrointestinal losses (e.g., diarrhea, vomiting, nasogastric suction), renal losses (e.g., overdiuresis, diabetes insipidus), and sequestration of extracellular fluid (e.g., third spacing, as seen in acute pancreatitis; see Box 29.1). Common conditions that cause effective intravascular volume contraction include heart failure and liver failure. In these conditions, the absolute blood volume is increased, yet arterial blood flow to the kidneys is reduced, leading to prerenal azotemia. A more detailed narrative on prerenal AKI is presented in Chapter 28 on the pathophysiology of AKI.

Intrinsic AKI is frequently categorized based on the anatomic location of primary renal injury, including the vasculature, glomeruli, interstitium, and tubules (see Table 29.1). Diseases that affect the renal vasculature range from complete thrombosis of the renal artery or vein, which are uncommon events, atheroembolic disease typically following instrumentation of renal or extrarenal vasculature, to vasculitides that affect small renal vessels, including the glomeruli. Such vasculitides may be confined to the kidney but are commonly systemic conditions with extrarenal manifestations. Acute interstitial nephritis (AIN) most often results from an idiosyncratic allergic response to a myriad of different pharmacologic agents, most commonly to antibiotics (e.g., methicillin and other penicillins, cephalosporins, sulfonamides, quinolones) or nonsteroidal antiinflammatory drugs (NSAIDs; e.g., ibuprofen).[43] Proton pump inhibitors have more recently been associated with impaired kidney function, hypothesized to be related, at least in part, to AIN.[44–46] A multitude of other clinical conditions, including bacterial infection, can also lead to AIN. Systemic allergic signs such as fever, rash, and eosinophilia are often present in antibiotic-associated AIN, but not usually present in NSAID-related AIN.

The most common form of intrinsic AKI is acute tubular necrosis (ATN). The causes of ATN are broadly categorized as ischemic, septic, and nephrotoxic. Prolonged prerenal azotemia and hypotension, even if short-lived, can lead to necrosis of tubular epithelial cells and ATN. Nephrotoxins leading to ATN can be extrinsic, such as iodinated contrast, antibiotics, and chemotherapeutic agents, or intrinsic, such as myoglobin, hemoglobin, and intratubular crystals (Table 29.3). Sepsis-associated AKI, although previously attributed to ischemia reperfusion injury, is now believed to have a more complex pathogenesis and may develop in the absence of overt hypotension. These conditions, including their pathogenesis and pathophysiology, are discussed in detail in Chapter 28.

Postrenal (obstructive) AKI is characterized by blockage to the flow of urine. Obstruction, intrinsic or extrinsic, can occur at any point from the renal collecting system to the urethra (see Box 29.2). The development of postrenal AKI requires bilateral obstruction or unilateral obstruction in a patient with a solitary kidney or in the setting of CKD. The most common causes of postrenal AKI are functional or structural obstruction of the bladder neck due to prostatic conditions, anticholinergic agents, and neurogenic bladder. Additional causes of obstructive AKI include bilateral ureteral calculi, fibrosis, or blood clots, sloughed renal papillae (i.e., renal papillary necrosis), genitourinary malignancies, external compression from tumors or hemorrhage, and retroperitoneal fibrosis. A detailed description of renal obstruction is provided in Chapter 37.

EVALUATION OF ACUTE KIDNEY INJURY

The assessment of the patient with AKI requires a meticulous history and physical examination, comprehensive review of medical records, evaluation of urinary findings, including the urinary sediment, review of laboratory tests, renal imaging and, when appropriate, kidney biopsy[47] (Table 29.4). Analysis

Table 29.3 Major Causes of Endogenous and Exogenous Toxins Causing Acute Tubular Necrosis

Endogenous Toxins	Exogenous Toxins
Myoglobulinuria • Muscle breakdown—trauma, compression, electric shock, hypothermia, hyperthermia, seizures, exercise, burns • Metabolic—hypokalemia, hypophosphatemia • Infections—tetanus, influenza, • Toxins—isopropyl alcohol, ethanol, ethylene glycol, toluene, snake and insect bites, cocaine, heroin • Drugs—HMG-CoA reductase inhibitors (statins), amphetamines, fibrates • Inherited disease—deficiency of myophosphorylase, phosphofructokinase, carnitine palmityltransferase • Autoimmune—polymyositis, dermatomyositis	Antibiotics • Aminoglycosides • Amphotericin B • Antiviral agents—acyclovir, cidofovir, indinavir, foscarnet, tenofovir • Pentamidine • Vancomycin
Hemoglobinuria • Mechanical—prosthetic valves, microangiopathic hemolytic anemia, extracorporeal circulation • Drugs—hydralazine, methyldopa • Chemicals—benzene, arsine, fava beans, glycerol, phenol • Immunologic—transfusion reaction • Genetic—G6PD deficiency, PNH	Chemotherapy • Cisplatin • Ifosfamide • Plicamycin • 5-Fluorouracil • Cytarabine • 6-Thioguanine • Methotrexate
Intratubular obstruction from crystalluria or paraproteins • Tumor lysis syndrome • HGPT deficiency	Calcineurin inhibitors • Cyclosporine • Tacrolimus
Multiple myeloma • Oxalate (ethylene glycol)	Organic solvents • Toluene • Ethylene glycol
	Poisons
	Miscellaneous • Radiocontrast agents • Intravenous immune globulin • Nonsteroidal antiinflammatory drugs • Oral phosphate bowel preparations

G6PD, Glucose-6-phosphate dehydrogenase; *HGPT,* Hypoxanthine-guanine phosphoribosyltransferase; *HMG-CoA,* 5-hydroxy-3-methylglutaryl-coenzyme A; *PNH,* paroxysmal nocturnal hemoglobinuria.

of serum creatinine concentration over time is invaluable for differentiating acute from chronic kidney disease and identifying the timing of events that precipitated the acute decline in kidney function. The presence of an acute process is easily confirmed if review of laboratory records reveals a sudden rise in blood urea nitrogen (BUN) level and serum creatinine concentration from previously stable baseline values. Spurious causes of elevated BUN and serum creatinine levels must be excluded before a diagnosis of AKI is made. When prior BUN and serum creatinine measurements are not available, key findings that suggest that a chronic process is present include physical manifestations of hyperparathyroidism (e.g., resorption of distal phalangeal tufts, lateral aspect of clavicles), band keratopathy, so-called half-and-half nails, and small echogenic kidneys on radiographic imaging. Enlarged kidneys do not necessarily rule out a chronic process because diabetic nephropathy, HIV-associated nephropathy, amyloidosis, and polycystic kidney disease are characterized by enlarged kidney size, even with moderate to advanced CKD. Anemia is a less useful differentiating feature because it is often present in both AKI and CKD. Once the presence of AKI has been confirmed, attention should focus on patient, urine, laboratory, and radiographic assessments to help distinguish among prerenal, intrinsic, and postrenal processes, permit identification of the cause of AKI, and guide treatment.

CLINICAL ASSESSMENT OF THE PATIENT

Prerenal AKI should be suspected in clinical settings associated with intravascular volume depletion, including hemorrhage, excessive gastrointestinal, urinary, or insensible fluid losses and severe burns, or with reduced effective arterial blood volume due to congestive heart failure, liver disease, or nephrotic syndrome. The risk of intravascular volume depletion is increased in comatose, sedated, or obtunded patients and in patients with restricted access to salt and water. Clinical clues to a prerenal cause of AKI on history include patient report of excessive thirst, orthostatic light-headedness or dizziness, significant diarrhea and/or vomiting, diuretic use, and recent use of medications that alter intrarenal hemodynamics, including NSAIDs, as well as inhibitors of the renin-angiotensin axis, such as direct renin inhibitors, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin receptor blockers (ARBs).

Findings suggestive of volume depletion on physical examination may include orthostatic hypotension (postural

Table 29.4 Useful Clinical Features, Urinary Findings, and Confirmatory Tests in the Differential Diagnosis of Acute Kidney Injury

Cause of Acute Kidney Injury	Some Suggestive Clinical Features	Typical Urinalysis	Some Confirmatory Tests
Prerenal azotemia	Evidence of true volume depletion (thirst, postural or absolute hypotension and tachycardia, low jugular venous pressure, dry mucous membranes and axillae, weight loss, fluid output >input) or decreased effective circulatory volume (e.g., heart failure, liver failure), treatment with NSAID, diuretic, or ACE inhibitor or ARB	Hyaline cases FE_{Na} <1% U_{Na} <10 mmol/L SG >1.018	Occasionally requires invasive hemodynamic monitoring; rapid resolution of AKI with restoration of renal perfusion
Diseases Involving Large Renal Vessels			
Renal artery thrombosis	History of atrial fibrillation or recent myocardial infarction, nausea, vomiting, flank or abdominal pain	Mild proteinuria Occasionally RBCs	Elevated LDH with normal transaminase levels, renal arteriogram, MAG-3 renal scan, MRA[a]
Atheroembolism	Usually age >50 years, recent manipulation of aorta, retinal plaques, subcutaneous nodules, palpable purpura, livedo reticularis	Often normal Eosinophiluria Rarely casts	Eosinophilia, hypocomplementemia, skin biopsy, renal biopsy
Renal vein thrombosis	Evidence of nephrotic syndrome or pulmonary embolism, flank pain	Proteinuria, hematuria	Inferior venocavogram, Doppler flow studies, MRV[a]
Diseases of Small Renal Vessels and Glomeruli			
Glomerulonephritis or vasculitis	Compatible clinical history (e.g., recent infection), sinusitis, lung hemorrhage, rash or skin ulcers, arthralgias, hypertension, edema	RBC or granular casts, RBCs, white blood cells, proteinuria	Low complement levels; positive antineutrophil cytoplasmic antibodies, antiglomerular basement membrane antibodies, antistreptolysin O antibodies, anti-DNAse, cryoglobulins; renal biopsy
HUS, TTP	Compatible clinical history (e.g., recent gastrointestinal infection, cyclosporine, anovulants), pallor, ecchymoses, neurologic findings	May be normal, RBCs, mild proteinuria, rarely RBC or granular casts	Anemia, thrombocytopenia, schistocytes on peripheral blood smear, low haptoglobin, increased LDH, renal biopsy
Malignant hypertension	Severe hypertension with headaches, cardiac failure, retinopathy, neurologic dysfunction, papilledema	May be normal, RBCs, mild proteinuria, rarely RBC casts	LVH by echocardiography or EKG, resolution of AKI with BP control
Ischemic or Nephrotoxic Acute Tubular Necrosis			
Ischemia	Recent hemorrhage, hypotension, surgery, often in combination with vasoactive medication (e.g., ACE inhibitor, NSAID)	Muddy brown granular or tubular epithelial cell casts FE_{Na} >1%, U_{Na} >20 mmol/L, SG ≈ 1.010	Clinical assessment and urinalysis usually inform diagnosis.
Exogenous toxin	Recent contrast-enhanced procedure; nephrotoxic medications; certain chemotherapeutic agents often with coexistent volume depletion, sepsis or chronic kidney disease	Muddy brown granular or tubular epithelial cell cases FE_{Na} >1%, U_{Na} >20 mmol/L, SG ≈ 1.010	Clinical assessment and urinalysis usually inform diagnosis.
Endogenous toxin	History suggestive of: Rhabdomyolysis (coma, seizures, drug abuse, trauma) Hemolysis (recent blood transfusion) Tumor lysis (recent chemotherapy) Myeloma (bone pain) or ethylene glycol ingestion	Urine supernatant tests positive for heme in absence of red cells; urine supernatant pink and tests positive for heme in absence of red cells; urate crystals, dipstick-negative proteinuria, oxalate crystals, respectively	Hyperkalemia, hyperphosphatemia, hypocalcemia, increased CK, myoglobin; hyperkalemia, hyperphosphatemia, hypocalcemia, hyperuricemia and free circulating hemoglobin; hyperuricemia, hyperkalemia, hyperphosphatemia (for tumor lysis); circulating or urinary monoclonal protein (for myeloma); toxicology screen, acidosis, osmolal gap (for ethylene glycol)

Table 29.4 Useful Clinical Features, Urinary Findings, and Confirmatory Tests in the Differential Diagnosis of Acute Kidney Injury (Cont'd)

Cause of Acute Kidney Injury	Some Suggestive Clinical Features	Typical Urinalysis	Some Confirmatory Tests
Diseases of the tubulointerstitium—allergic interstitial nephritis	Recent ingestion of drug and fever, rash, loin pain, or arthralgias	White blood cell casts, white blood cells (frequently eosinophiluria), RBCs, rarely RBC casts, proteinuria (occasionally nephritic)	Systemic eosinophilia, renal biopsy
Acute bilateral pyelonephritis	Fever, flank pain and tenderness, toxic state	Leukocytes, occasionally white blood cell casts, RBCs, bacteria	Urine and blood cultures
Postrenal AKI	Abdominal and flank pain, palpable bladder	Frequently normal, hematuria if stones, prostatic hypertrophy	Plain abdominal x-ray, renal ultrasonography, postvoid residual bladder volume, computed tomography, retrograde or antegrade pyelography

[a]Contrast-enhanced magnetic resonance angiography and magnetic resonance venography should be used with extreme caution in patients with AKI.

ACE, Angiotensin-converting enzyme; *AKI,* acute kidney injury; *ARB,* angiotensin receptor blocker; *BP,* blood pressure; *CK,* creatine kinase; *EKG,* electrocardiogram; FE_{Na}, fractional excretion of sodium; *HUS,* hemolytic-uremic syndrome; *LDH,* lactate dehydrogenase; *LVH,* left ventricular hypertrophy; *MRA,* magnetic resonance angiography; *MRV,* magnetic resonance venography; *NSAID,* nonsteroidal antiinflammatory drug; *RBC,* red blood cell; *SG,* specific gravity; *TTP,* thrombotic thrombocytopenic purpura; U_{Na}, urine sodium.

fall in diastolic blood pressure >10 mm Hg) and tachycardia (postural increase in heart rate >10 beats/min), reduced jugular venous pressure, diminished skin turgor, dry mucous membranes, and the absence of axillary sweat. However, overt signs and symptoms of hypovolemia do not usually manifest until the extracellular fluid volume has fallen by more than 10% to 20%. In addition, in patients with heart failure, liver disease, or nephrotic syndrome, renal hypoperfusion may be present, despite total body volume overload. Findings on physical examination of peripheral edema, pulmonary vascular congestion, pleural effusion, cardiomegaly, the presence of an S3 heart sound, elevated jugular venous pressure, or hepatic congestion may point to a state of reduced cardiac output and decreased effective intravascular volume. The presence of acute or chronic liver disease is suggested by evidence of icterus, ascites, splenomegaly, palmar erythema, telangiectasia, and caput medusa. In select critically ill patients, invasive hemodynamic monitoring using central venous or pulmonary artery catheters or ultrasonography of the heart and central veins may assist in assessing intravascular volume status. A definitive diagnosis of prerenal AKI is usually based on the prompt resolution of AKI after restoration of renal perfusion. In patients with underlying systolic heart failure, restoration of renal perfusion may be challenging and often requires the use of inotropic support.

There is a high likelihood of ischemic ATN if AKI follows a period of severe renal hypoperfusion, and the impairment in kidney function persists or worsens, despite restoration of renal perfusion. It should be noted, however, that significant hypotension is evident in fewer than 50% of patients with postsurgical ATN.[48] Although septic shock is a common cause of ATN, ATN may also develop in sepsis in the absence of overt hypotension.[48,49] The diagnosis of nephrotoxic ATN requires a comprehensive review of all clinical, pharmacy, nursing, radiographic, and procedural notes for evidence of the administration of nephrotoxic agents. Pigment-induced ATN may be suspected if the clinical assessment reveals risk factors for rhabdomyolysis (e.g., seizures, excessive exercise, alcohol or drug abuse, treatment with statins, prolonged immobilization, limb ischemia, crush injury) or hemolysis, as well as select signs and symptoms of the former (e.g., muscle tenderness, weakness, evidence of trauma or prolonged immobilization).[50–53]

Although most AKI is prerenal or ischemic, nephrotoxic, or septic ATN, patients should be carefully evaluated for other intrinsic renal parenchymal processes because their management and prognosis may differ substantially. Flank pain may be a prominent symptom of acute renal artery or renal vein occlusion, renal infarction (from systemic emboli), acute pyelonephritis and, rarely, necrotizing glomerulonephritis.[54–56] Interstitial edema leading to distention of the renal capsule and flank pain may be seen in up to one-third of patients with AIN.[57] Dermatologic examination can be highly informative because a maculopapular rash may accompany allergic interstitial nephritis: subcutaneous nodules, livedo reticularis, digital ischemia, and palpable purpura may suggest atheroembolism or vasculitis; a malar (butterfly) rash may be associated with systemic lupus erythematosus; and impetigo or needle tracks from intravenous (IV) drug use may underlie infection-associated glomerulonephritis. An ophthalmologic examination is useful to assess for signs of atheroembolism; hypertensive or diabetic retinopathy; the keratitis, uveitis, and iritis of autoimmune vasculitides; icterus associated with chronic liver disease; and the rare but nevertheless pathognomonic band keratopathy of hypercalcemia and flecked retina of hyperoxalemia. Uveitis may also

be an indicator of coexistent allergic interstitial nephritis, sarcoidosis, and the tubulointerstitial nephritis and uveitis (TINU) syndrome.[58] Examination of the ears, nose, and throat may reveal conductive deafness and mucosal inflammation or ulceration suggestive of necrotizing granulomatous vasculitis or the neural deafness caused by aminoglycoside toxicity. Respiratory failure, particularly if associated with hemoptysis, suggests the presence of a pulmonary-renal syndrome, and ascites and other stigmata of severe chronic liver disease suggest the possibility of hepatorenal syndrome (HRS). Cardiovascular assessment may reveal marked elevation in systemic blood pressure suggesting malignant hypertension or scleroderma or may demonstrate a new arrhythmia or murmur suggesting a potential source of thromboemboli or subacute bacterial endocarditis (acute glomerulonephritis), respectively. Chest or abdominal pain and reduced pulses in the lower limbs should suggest aortic dissection or, rarely, Takayasu arteritis. Abdominal pain and nausea are frequent clinical correlates of atheroembolic disease, commonly in patients who have recently undergone angiographic evaluation, particularly in the presence of widespread atherosclerotic disease. The presence of a tensely distended abdomen may indicate the presence of abdominal compartment syndrome and should prompt transduction of bladder pressure.[59] Pallor and recent bruising are important clues to the thrombotic microangiopathies, and the combination of bleeding and fever should raise the possibility of AKI resulting from viral hemorrhagic fevers. A recent jejunoileal bypass may be a vital clue to oxalosis, a rare but reversible cause of AKI following bariatric surgery.[60] Hyperreflexia and asterixis often portend the development of uremic encephalopathy or may, in the presence of focal neurologic signs, suggest a diagnosis of thrombotic microangiopathy (i.e., hemolytic-uremic syndrome [HUS] or thrombotic thrombocytopenic purpura [TTP]; see Chapter 34).

Postrenal AKI may be asymptomatic if obstruction to the drainage of urine develops gradually. Although anuria will be seen in complete obstruction, urine volume may be normal or even increased in the setting of partial obstruction. A pattern of fluctuating urine output may also be seen in some patients with partial obstruction. Suprapubic or flank pain may be the presenting complaint if there is acute distention of the bladder or renal collecting system and capsule, respectively. Colicky flank pain radiating to the groin suggests acute ureteral obstruction, most commonly from urinary stone disease. Prostatic disease should be suspected in older men with a history of nocturia, urinary frequency, urgency or hesitancy, and an enlarged prostate on rectal examination. Urinary retention may be exacerbated acutely in such patients by medications with anticholinergic properties, such as antihistamines or antidepressants. Rectal or pelvic examination may reveal obstructing tumors in female patients. Neurogenic bladder is a likely diagnosis in patients with spinal cord injury or autonomic insufficiency and should be suspected in patients with long-standing diabetes mellitus. Bladder distention may be evident on abdominal percussion and palpation in patients with bladder neck or urethral obstruction. Definitive diagnosis of postrenal AKI usually relies on examination of the postvoid bladder volume and radiographic evaluation of the upper urinary tract and is confirmed by improvement in kidney function following relief of the obstruction.

URINE ASSESSMENT

Urine volume is rarely helpful in distinguishing various forms and causes of AKI. Anuria can be seen with complete urinary tract obstruction but can also be seen with severe prerenal or intrinsic renal disease (e.g., renal artery occlusion, severe proliferative glomerulonephritis or vasculitis, bilateral cortical necrosis). Patients with partial urinary tract obstruction may present with polyuria caused by secondary impairment of urine-concentrating mechanisms.

Assessment of the urine is essential in patients with AKI and is an inexpensive and useful diagnostic tool.[61–63] Measured urine specific gravity above 1.015 to 1.020 often accompanies prerenal AKI, although impaired urinary concentration may be present in patients with underlying CKD or as a result of diuretic therapy. Acute glomerulonephritis may also present with concentrated urine. Isosthenuria (a urine specific gravity of 1.010, similar to that of plasma) is characteristic of ATN. Hematuria on dipstick assessment may result from urologic trauma from catheterization, urologic disease, interstitial nephritis, acute glomerulonephritis, atheroembolic disease, renal infarction, or pigment (hemoglobinuric or myoglobinuric) nephropathy. The latter are suggested when the dipstick test for blood is strongly positive but there are few or no red blood cells seen on microscopic examination of the urinary sediment.

Examination of the urinary sediment of a centrifuged urine specimen complements the dipstick analysis and is highly valuable for distinguishing among the various forms of AKI (Box 29.3). The sediment should be inspected for the presence of cells, casts, and crystals. In prerenal AKI, the urine sediment is typically bland (i.e., devoid of cells or casts) but may contain transparent hyaline casts. Hyaline casts are formed in concentrated urine from normal urinary constituents, principally Tamm-Horsfall protein secreted by epithelial cells of the loop of Henle. Postrenal AKI may also present with a bland urine sediment, although hematuria is common in patients with intraluminal obstruction (e.g., stones, sloughed papilla, blood clot) or prostatic disease. Renal tubular epithelial cells, epithelial cell casts, and pigmented, muddy brown granular casts are characteristic of ischemic or nephrotoxic ATN. These characteristic findings of ATN may be found in association with microscopic hematuria and mild tubular proteinuria (<1 g/day). Casts may be absent in approximately 20% to 30% of patients with ischemic or nephrotoxic ATN and are not a requisite for diagnosis[61,64]; however, semiquantitative scoring systems have been developed to assess the presence of epithelial cells and casts in patients with AKI to assist in the diagnosis of ATN and correlate with the clinical course.[62,65,66]

Red blood cell (RBC) casts are almost always indicative of acute glomerular disease but may be observed, albeit rarely, in AIN. Dysmorphic RBCs, best seen using phase contrast microscopy, are a more common urinary finding in patients with glomerular injury but are a less specific finding than RBC casts. Urine sediment abnormalities vary in diseases involving preglomerular blood vessels, such as HUS, TTP, atheroembolic disease, and vasculitis involving medium-sized or large vessels, and range from benign to overtly nephritic. White blood cell casts and nonpigmented granular casts suggest interstitial nephritis, whereas broad granular casts are characteristic of CKD and probably reflect interstitial fibrosis and dilation of

Box 29.3 Urine Sediment in the Differential Diagnosis of Acute Kidney Injury

Normal or few red blood cells or white blood cells
- Prerenal azotemia
- Arterial thrombosis or embolism
- Preglomerular vasculitis
- HUS, TTP
- Scleroderma crisis
- Postrenal AKI

Renal tubular epithelial cells and granular casts
- Acute tubular necrosis

Dysmorphic red blood cells and red blood cell casts
- Glomerulonephritis or vasculitis
- Malignant hypertension
- Rarely interstitial nephritis

White blood cell and white blood cell casts
- Acute interstitial nephritis or exudative glomerulonephritis
- Severe pyelonephritis
- Marked leukemic or lymphomatous infiltration

Eosinophiluria (>5%)
- Allergic interstitial nephritis (antibiotics >> NSAIDs)
- Atheroembolism

Crystalluria
- Acute urate nephropathy
- Calcium oxalate (ethylene glycol intoxication)
- Acyclovir
- Indinavir
- Sulfonamides
- Methotrexate

AKI, Acute kidney injury; *HUS,* hemolytic-uremic syndrome; *NSAIDs,* nonsteroidal antiinflammatory drugs; *TTP,* thrombotic thrombocytopenic purpura.

tubules. Eosinophiluria (from 1%–50% of urine leukocytes) is a common finding (90%) in drug-induced allergic interstitial nephritis.[67,68] However, eosinophiluria has poor sensitivity and specificity for the diagnosis of AIN, with eosinophiluria of 1% to more than 5% occurring in a variety of other diseases, including atheroembolization, ischemic and nephrotoxic AKI, proliferative glomerulonephritis, pyelonephritis, cystitis, and prostatitis. In a series of 566 patients who had urinary eosinophil testing and renal histology from kidney biopsy, eosinophiluria only had a 31% sensitivity and 68% specificity for the diagnosis of interstitial nephritis.[69] Uric acid crystals (pleomorphic) may be seen in the urine in prerenal AKI but should raise the possibility of acute urate nephropathy if seen in abundance. Oxalate crystalluria (needle- or dumbbell-shaped monohydrate crystals or envelope-shaped dihydrate crystals) may suggest a diagnosis of ethylene glycol toxicity.[70] A variety of other crystals may be seen in other medication-associated crystal nephropathies.[71–73]

Increased urinary protein excretion, characteristically less than 1 g/day, is a common finding in ischemic or nephrotoxic ATN and reflects both failure of injured proximal tubule cells to reabsorb normally filtered protein and excretion of cellular debris (tubular proteinuria). Proteinuria greater than 1 g/day suggests injury to the glomerular ultrafiltration barrier (glomerular proteinuria) or excretion of light chains.[74,75] The latter are not detected by conventional dipsticks, which detect albumin, and must be sought by other means (e.g., sulfosalicylic acid test). Heavy proteinuria is also a frequent finding (80%) in patients with allergic interstitial nephritis triggered by NSAIDs. In addition to acute interstitial inflammation, these patients have a glomerular lesion that is almost identical to that of minimal-change disease.[76] A similar syndrome has been reported in patients receiving other agents, such as ampicillin, rifampin, and interferon alfa.[77,78] Hemolysis and rhabdomyolysis may often be differentiated by inspection of plasma, which is characteristically pink in hemolysis, but clear in rhabdomyolysis.

Analysis of urine biochemical parameters may be helpful in differentiating between prerenal and intrinsic ischemic or nephrotoxic ATN (Table 29.5). Sodium is usually avidly reabsorbed from the glomerular filtrate in patients with prerenal AKI as a consequence of renal adrenergic activation, stimulation of the renin-angiotensin-aldosterone system (RAAS), suppression of atrial natriuretic peptide (ANP) secretion, and local changes in peritubular hemodynamics. In contrast, Na^+ reabsorption is impaired in ATN as a result of injury to the renal tubular epithelium. Renal sodium handling can be assessed based on the urinary sodium concentration (U_{Na}), with values of less than 10 mmol/L commonly seen in prerenal disease compared with more than 20 mmol/L in ATN. Normalizing sodium excretion to creatinine provides a more sensitive index. The fractional excretion of sodium (FE_{Na}) is the ratio between urine sodium excretion ($U_{Na} \times V$, where U_{Na} is the urine sodium concentration and V is the urine volume) and the filtered load of sodium (calculated as $P_{Na} \times CrCl$, where P_{Na} is the plasma sodium concentration and CrCl is the creatinine clearance, which can be calculated as $[(U_{Cr} \times V)/P_{Cr}]$ where V is the urine volume and $[U_{Cr}]$ and $[P_{Cr}]$ are the urine and plasma creatinine concentrations, respectively. Because urine volume is in both the numerator and denominator of this ratio, the FE_{Na} can be calculated as $[(U_{Na} \div P_{Na})/(U_{Cr} \div P_{Cr})] \times 100$ using an untimed (spot) urine sample and simultaneous serum sodium and creatinine measurements; this is usually less than 1% (frequently, <0.5%) in the setting of prerenal azotemia, whereas it is typically more than 2% in patients with ischemic or nephrotoxic AKI.

The utility of the FE_{Na} is limited in a variety of clinical settings. Values greater than 1% are not uncommon in the setting of prerenal AKI in patients receiving diuretics, those with metabolic alkalosis and bicarbonaturia (in whom Na^+ is excreted with HCO_3^- to maintain electroneutrality), in the presence of adrenal insufficiency, and in the setting of underlying CKD.[79–81] Conversely, an FE_{Na} less than 1% may be observed in the setting of ATN, particularly in the settings of iodinated contrast administration, rhabdomyolysis, and sepsis, although it has been reported in 15% or more of patients with ATN from a variety of other causes, including ischemia, burns, and exposure to selected nephrotoxins.[79,80,82–84]

It has been postulated that a low FE_{Na} value reflects a milder degree of intrinsic renal injury in which epithelial cell damage is probably localized to the corticomedullary junction and outer medulla, with relative preservation of function in other Na^+-transporting segments, and may represent a transition state between prerenal azotemia and ATN. It should be recognized that an FE_{Na} value of less than 1% is not abnormal and reflects normal sodium homeostasis in

Table 29.5 Urine Indices Used in the Differential Diagnosis of Prerenal Acute Kidney Injury and Acute Tubular Necrosis

Diagnostic Index	Prerenal AKI	ATN
Fractional excretion of sodium (%)	<1[a]	>2[a]
Urine sodium concentration (mmol/L)	<20	>40
Urine creatinine-to-plasma creatinine ratio	>40	<20
Urine urea nitrogen-to-plasma urea nitrogen ratio	>8	<3
Urine specific gravity	>1.018	~1.010
Urine osmolality (mOsm/kg H_2O)	>500	~300
Plasma BUN-to-creatinine ratio	>20	<10-15
Renal failure index, $U_{Na}/(U_{Cr}/P_{Cr})$	<1	>1
Urine sediment	Hyaline casts	Muddy brown granular casts

[a]FE_{Na} may be >1% in prerenal AKI associated with diuretic use and/or the setting of bicarbonaturia or chronic kidney disease; FE_{Na} often <1% in acute tubular necrosis caused by radiocontrast or rhabdomyolysis.

AKI, Acute kidney injury; *ATN*, acute tubular necrosis; *BUN,* blood urea nitrogen; P_{Cr}, plasma creatinine; U_{Cr}, urine creatinine; U_{Na}, urine sodium.

patients on a moderate- to low-sodium diet. The FE_{Na} is also often less than 1% in AKI caused by urinary tract obstruction, glomerulonephritis, and diseases of the renal vasculature; other parameters must be used to distinguish these conditions from prerenal AKI.

A variety of other indices has also been proposed to differentiate between causes of AKI. The renal failure index, calculated as $U_{Na}/(U_{Cr} \div P_{Cr})$ provides comparable information to the FE_{Na} because clinical variations in serum Na^+ concentration are relatively small. The fractional excretion of urea (FE_{urea}) has been proposed as an alternative to the FE_{Na}, with particular utility in patients on diuretic therapy. Values of FE_{urea} calculated as $([U_{urea} \div P_{urea}]/[U_{Cr} \div P_{Cr}] \times 100)$ less than 35% are suggestive of a prerenal state.[85–87] Similarly, indices of urinary concentrating ability, such as urine specific gravity, urine osmolality, urine-to-plasma creatinine or urea ratios, and serum urea nitrogen-to-creatinine ratio are of limited value in differentiating between prerenal and intrinsic AKI. This is particularly true for older patients, in whom urine concentrating mechanisms are frequently impaired while mechanisms for Na^+ reabsorption are typically preserved.

BLOOD AND LABORATORY FINDINGS

The pattern and timing of changes of BUN and serum creatinine levels often provide clues to the cause of AKI. Enhanced tubular reabsorption of filtered urea, in parallel with sodium and water reabsorption in prerenal states, commonly leads to a disproportionate elevation in BUN relative to serum creatinine levels (ratio >20:1). Conversely, with intrinsic AKI, the increase in BUN level usually parallels the rise in serum creatinine level, maintaining a ratio of about 10:1. However, severe malnutrition and low dietary protein intake blunt the rise in BUN and creatinine levels, whereas gastrointestinal bleeding, steroid therapy, and hypercatabolic states may lead to increases in the BUN level that do not reflect prerenal physiology. In addition, aggressive volume resuscitation may rapidly expand the volume of distribution of urea and creatinine and may also obscure the acute rise in serum creatinine level. Sepsis and other forms of critical illness have also been associated with decreased creatinine generation.[88,89] The serum creatinine level typically begins to rise within 24 to 48 hours when ATN results from an ischemic insult. Although the clinical course can be highly variable, the serum creatinine level will generally peak within 7 to 10 days and, depending on the severity of the insult and underlying comorbid illnesses, AKI will resolve to varying degrees over the ensuing 1 to 2 weeks. Following iodinated contrast exposure, the peak in serum creatinine level generally occurs within 5 to 7 days. The time course of nephrotoxic ATN caused by aminoglycoside antibiotics or cisplatin is more variable, often with a delayed onset of AKI (7–10 days).

Additional clues to the diagnosis can be obtained from biochemical and hematologic tests. The presence of marked hyperkalemia, hyperuricemia, and hyperphosphatemia point to cell lysis, which, in the setting of elevated creatine kinase levels and hypocalcemia, strongly suggests rhabdomyolysis.[90,91] Biochemical signs of cell lysis with very high levels of uric acid, normal or mildly elevated creatine kinase levels, and a urine uric acid-to-creatinine ratio greater than 1.0 are suggestive of acute urate nephropathy and tumor lysis syndrome.[92,93] Severe hypercalcemia can precipitate AKI, commonly in the form of prerenal AKI from concomitant hypovolemia and renal vasoconstriction. AKI associated with a widening of both the serum anion ($Na^+ - [HCO_3^- + Cl^-]$) and osmolal (measured serum osmolality minus calculated osmolality) gaps suggests a diagnosis of ethylene glycol toxicity and should prompt a search for urine oxalate crystals. Severe anemia in the absence of hemorrhage may reflect the presence of hemolysis, multiple myeloma, or thrombotic microangiopathy (e.g., HUS, TTP, toxemia, disseminated intravascular coagulation, accelerated hypertension, systemic lupus erythematosus [SLE], scleroderma, radiation injury). Other laboratory findings suggestive of thrombotic microangiopathy include thrombocytopenia, dysmorphic RBCs on peripheral blood smear, low circulating haptoglobin concentrations, and elevated circulating concentrations of lactate dehydrogenase. Systemic eosinophilia suggests allergic interstitial nephritis but may also be a prominent feature in other diseases, such as atheroembolic disease and eosinophilic

granulomatosis with polyangiitis (Churg-Strauss disease). Depressed serum complement and high titers of antiglomerular basement membrane antibodies, antineutrophil cytoplasmic antibodies, antinuclear antibodies, circulating immune complexes, or cryoglobulins are useful diagnostic tools in patients with suspected glomerulonephritis or systemic vasculitis.

NOVEL BIOMARKERS OF KIDNEY INJURY

A number of novel biomarkers of kidney injury have been evaluated for potential roles in the early identification, differential diagnosis, and prognosis of AKI, including serum cystatin C, NGAL, KIM-1, IL-18, L-FABP, TIMP-2 and IGFBP7 among others (see earlier).[17–24] Although most of these biomarkers are not available for routine clinical use, they have the potential to provide an earlier diagnosis of intrinsic AKI, differentiate volume-responsive (prerenal) AKI from intrinsic disease, and provide prognostic information regarding the clinical course of an episode of AKI.

Cystatin C

Cystatin C is a 13-kDa protein that is filtered by the glomerulus and completely reabsorbed and degraded by the proximal tubule. Cystatin C has been validated as an alternative marker of glomerular filtration.[94,95] As a result of its shorter serum half-life, serum cystatin C concentrations change more rapidly than serum creatinine levels in response to changes in kidney function, allowing changes in serum cystatin C to be detected sooner than changes in serum creatinine following the onset of AKI.[96] Under normal circumstances, urinary cystatin C is virtually undetectable; however, following tubular injury, tubular reabsorption of filtered cystatin is diminished, and urinary cystatin C can be detected, raising the possibility of its use as an early marker of tubular injury.[97]

Neutrophil Gelatinase-Associated Lipocalin

NGAL is a 25-kDa protein whose expression by renal tubular epithelial cells is markedly upregulated following ischemic or nephrotoxic kidney injury.[98,99] NGAL is believed to enhance the trafficking of iron-siderophore complexes, enhancing the delivery of iron, upregulating hemeoxygenase-1, reducing apoptosis, and increasing the normal proliferation of renal tubule epithelial cells.[100] Urine and plasma NGAL have been evaluated in numerous clinical settings as an early biomarker of tubular injury.[101–111] Initial studies in children undergoing cardiac surgery have demonstrated extremely high sensitivity and specificity for the identification of AKI, with an area under the receiver operating characteristic (ROC) curve of more than 0.99; however, these early results have not been reproduced across other clinical settings.

Kidney Injury Molecule 1

KIM-1 is a transmembrane protein whose expression is markedly upregulated in the proximal tubule following tubular injury.[112–115] The extracellular component of the KIM-1 protein is shed into the urine following tubular injury, permitting its potential use as a marker of tubular damage; however, the time course of peak KIM-1 expression in the urine is later than that seen with NGAL.[108,116–118] Moreover, more recent studies have demonstrated elevated levels of KIM-1 in non-AKI settings, including chronic kidney disease and renal cell carcinoma, which reduce its specificity for AKI.[119,120]

Interleukin 18

IL18 is a proinflammatory cytokine whose expression is increased in the kidney following ischemic and nephrotoxic injury.[121] Urinary IL-18 levels have been shown to rise within 6 hours following tubular injury, following cardiac surgery, and in critically ill patients.[110,111,122,123]

Liver Fatty Acid–Binding Protein

Despite its name, L-FABP is expressed in the proximal tubule.[124,125] Elevated L-FABP levels may be detected in the urine within 6 hours of ischemic or nephrotoxic injury, permitting its potential use as a marker of tubular injury.[118,126–128] In a meta-analysis of published studies, the sensitivity and specificity of urinary L-FABP for diagnosis of AKI were each approximately 75%.[129]

Tissue Inhibitor of Metalloproteinase 2 and Insulin-Like Growth Factor–Binding Protein 7

TIMP-2 and IGFBP7 are expressed in epithelial cells and act in an autocrine and paracrine manner to arrest cell cycle in AKI.[130–132] In three discovery cohorts comprising 522 patients, these two biomarkers were identified as having the highest discriminant ability among 340 candidate biomarkers of AKI.[22] In a subsequent validation study of 728 patients, this pair of biomarkers had an area under the ROC curve of 0.80, which was significantly better than the performance of other candidate biomarkers, including NGAL, KIM-1, IL-18, and L-FABP.[22] A combination test that includes TIMP-2 and IGFBP7 is available for commercial use.

RADIOLOGIC EVALUATION

Imaging of the abdomen is a highly useful adjunct to laboratory testing to determine the cause of AKI. In cases of suspected obstructive uropathy, postvoid residual volumes of more than 100 to 150 mL suggest a diagnosis of bladder outlet obstruction. Although plain films rarely provide definitive evidence of postrenal AKI, they may identify the presence of calcium-containing stones that can cause obstructive disease. Renal ultrasonography is the screening test of choice to assess cortical thickness, differences in cortical and medullary density, the integrity of the collecting system, and kidney size.[133] Although pelvicalyceal dilation is usual in cases of urinary tract obstruction (98% sensitivity), dilation may not be observed in the volume-depleted patient during the initial 1 to 3 days after obstruction, when the collecting system is relatively noncompliant, or in patients with obstruction caused by ureteric encasement or infiltration (e.g., retroperitoneal fibrosis, neoplasia).[134]

Alternatively, computed tomography (CT) may be used to visualize the kidneys and collecting system, although contrast administration should ideally be avoided in patients with AKI. Visualization of the collecting system may be suboptimal in the absence of contrast enhancement; however, unenhanced CT scans are useful for the identification of obstructing ureteral stones.[135,136] Ultrasonography and CT have essentially replaced the use of IV pyelography, which now has little or no role in the evaluation of AKI. Cystoscopic retrograde or percutaneous anterograde pyelography are useful tests for the precise localization of the site of obstruction and can be combined with placement of ureteral stents or percutaneous nephrostomy tubes to allow therapeutic decompression of

the urinary tract. Radionuclide scans have been proposed as useful for assessing renal blood flow, glomerular filtration, tubule function, and infiltration by inflammatory cells in AKI; however, these tests lack specificity and yield conflicting or poor results in controlled studies.[137,138] Magnetic resonance angiography (MRA) of the kidneys is extremely useful for detecting renal artery stenosis and has been used in the evaluation of acute renovascular crises.[139] However, given the association of gadolinium-based contrast administration with the development of nephrogenic systemic fibrosis, contrast-enhanced MRA is contraindicated in most patients with AKI.[140,141] Doppler ultrasonography and spiral CT are also useful in patients with suspected vascular obstruction; however, contrast angiography remains the gold standard for definitive diagnosis.

KIDNEY BIOPSY

Kidney biopsy in AKI is usually reserved for patients in whom prerenal and postrenal AKI have been excluded, and the cause of intrinsic AKI is unclear.[142,143] Kidney biopsy is particularly useful when clinical assessment, urinalysis, and laboratory investigation suggest diagnoses other than ischemic or nephrotoxic injury that may respond to specific therapy. Examples include anti–glomerular basement membrane disease and other forms of necrotizing glomerulonephritis, vasculitis, HUS and TTP, allergic interstitial nephritis, and myeloma cast nephropathy.

CAUSES OF ACUTE KIDNEY INJURY IN SPECIFIC CLINICAL SETTINGS

The differential diagnosis of AKI in several common clinical settings warrants special mention (Box 29.4).

ACUTE KIDNEY INJURY IN THE SETTING OF CANCER

There are several potential causes of AKI in the patient with cancer. Prerenal AKI is common in the setting of underlying malignancy and may be related to tumor- or chemotherapy-induced vomiting or diarrhea, reduced oral intake secondary to anorexia, the use of NSAIDs for pain management, and malignancy-associated hypercalcemia.[144,145]

Intrinsic AKI can be triggered by a variety of chemotherapeutic agents. Cisplatin is the classic chemotherapeutic medication associated with AKI.[146,147] The principal site of renal damage with cisplatin is the proximal tubule. The nephrotoxicity of cisplatin is dose-dependent, yet AKI can result from a single exposure. Electrolyte disturbances, including hypomagnesemia and hypokalemia, are common following cisplatin administration. Other platinum-containing chemotherapy agents, such as carboplatin and oxaliplatin, are less nephrotoxic than cisplatin but are not risk-free, particularly when high cumulative doses are administered. Ifosphamide, which has been used to treat germ cell tumors, sarcomas, other solid tumors, and occasionally lymphoma, is also associated with AKI in a dose-dependent fashion.[148–150] Methotrexate nephrotoxicity occurs following IV administration of high doses (>1 g/m^2), primarily as the result of precipitation of the drug and metabolites in the tubular lumen.[71,151,152] Risk factors for methotrexate nephrotoxicity include volume depletion and the presence of acidic urine. Direct tubular toxicity may also contribute to the development of AKI. Chemotherapeutic agents targeting vascular endothelial growth factor (VEGF) or the VEGF receptor, such as bevacizumab, and the tyrosine kinase inhibitor sunitinib, are associated with hypertension, proteinuria, thrombotic microangiopathy, and AKI.[153,154] Checkpoint inhibitors, such as nivolumab, may cause a variety of immune-related adverse events, including AIN.[155,156]

Renal parenchymal invasion by solid and hematologic cancers has been reported in 5% to 10% of autopsy studies but is an uncommon cause of AKI.[157,158] Infiltration of leukemic cells into the renal parenchyma can precipitate AKI and typically presents with hematuria, proteinuria, and enlarged kidneys on ultrasound imaging. Prompt diagnosis is important because the AKI may respond to chemotherapeutic intervention.

The tumor lysis syndrome, which is associated with hyperuricemia, hyperphosphatemia, and hypocalcemia, is a well-recognized cause of AKI in patients with cancer.[159,160] Tumor lysis syndrome occurs most commonly following the initiation of chemotherapy for patients with poorly differentiated, rapidly growing lymphoproliferative malignancies (e.g., Burkitt lymphoma, acute lymphoblastic or promyelocytic leukemia); however, this can occur spontaneously and in the setting of certain solid tumors that are highly sensitive to radiation and/or chemotherapy (e.g., testicular carcinoma). The Cairo-Bishop criteria, which include both laboratory and clinical criteria, have been used to provide a standard definition for the diagnosis of tumor lysis syndrome[160] (Box 29.5). AKI associated with the tumor lysis syndrome is thought to be triggered by direct tubular injury and luminal obstruction by uric acid and calcium phosphate crystals. Prophylactic therapy with aggressive volume administration and either xanthine oxidase inhibitors to inhibit uric acid synthesis or recombinant uricase to convert uric acid to allantoin has markedly reduced the incidence of this form of AKI.[161–164] Less common causes of AKI include tumor-associated glomerulonephritis and thrombotic microangiopathy induced by medications or irradiation. Chemotherapy-associated thrombotic microangiopathy is a well-recognized complication of several agents, including mitomycin C and gemcytabine.[165–167]

AKI is a common complication of multiple myeloma.[75,168] Causes of AKI in this setting include intravascular volume depletion, myeloma cast nephropathy, sepsis, hypercalcemia, ATN induced by drugs or tumor lysis during therapy, cryoglobulinemia, hyperviscosity syndrome, and plasma cell infiltration. Multiple myeloma may also result in impaired kidney function as the result of amyloidosis or light chain deposition disease; however, these usually present with proteinuria and a more subacute decline in kidney function. Myeloma cast nephropathy results from the binding of filtered immunoglobulin Bence-Jones proteins to Tamm-Horsfall glycoprotein, forming casts that obstruct the tubular lumen. Higher excretion rates of free light chains, volume depletion, and hypercalcemia are associated with higher risks for the development of myeloma cast nephropathy. Prompt treatment to lower the free light chain burden may result in recovery of kidney function. Studies of the effectiveness of plasmapheresis in the treatment of myeloma cast nephropathy have yielded conflicting results.[169–172] The use

Box 29.4 Major Causes of Acute Kidney Injury in Specific Clinical Settings

AKI in the Cancer Patient

Prerenal azotemia
- Hypovolemia (e.g., poor intake, vomiting, diarrhea)

Intrinsic AKI
- Exogenous nephrotoxins—chemotherapy, antibiotics, contrast agents
- Endogenous toxins—hyperuricemia, hypercalcemia, tumor lysis, paraproteins
- Other—radiation, HUS, TTP, glomerulonephritis, amyloid, malignant infiltration

Postrenal AKI
- Ureteric or bladder neck obstruction

AKI After Cardiac Surgery

Prerenal azotemia
- Hypovolemia (surgical losses, diuretics), cardiac failure, vasodilators

Intrinsic AKI
- Ischemic ATN (even in absence of hypotension)
- Atheroembolic disease after aortic manipulation, intraaortic balloon pump
- Pre- or perioperative administration of contrast agent
- Allergic interstitial nephritis induced by perioperative antibiotics

Postrenal AKI
- Obstructed urinary catheter, exacerbation of voiding dysfunction

AKI in Pregnancy

Prerenal azotemia—acute fatty liver of pregnancy with fulminate hepatic failure

Intrinsic AKI
- Preeclampsia or eclampsia
- Postpartum HUS, TTP
- HELLP syndrome
- Ischemia—postpartum hemorrhage, abruptio placentae, amniotic fluid embolus
- Direct toxicity of illegal abortifacients

Postrenal AKI—obstruction with pyelonephritis

AKI After Solid Organ or Bone Marrow Transplantation (BMT)

Prerenal azotemia
- Intravascular volume depletion (e.g., diuretic therapy)
- Vasoactive drugs (e.g., calcineurin inhibitors, amphotericin B)
- Hepatorenal syndrome, venoocclusive disease of liver (BMT)

Intrinsic AKI
- Postoperative ischemic ATN (even in absence of hypotension)
- Sepsis
- Exogenous nephrotoxins: aminoglycosides, amphotericin B, radiocontrast
- HUS, TTP (e.g., cyclosporine or myeloablative radiotherapy–related)
- Allergic tubulointerstitial nephritis

Postrenal AKI
- Obstructed urinary catheter

AKI and Pulmonary Disease (Pulmonary-Renal Syndrome)

Prerenal azotemia—diminished cardiac output complicating pulmonary embolism, severe pulmonary hypertension, or positive pressure mechanical ventilation

Intrinsic AKI
- Vasculitis—Goodpasture syndrome, ANCA-associated vasculitis, SLE, Churg-Strauss syndrome, polyarteritis nodosa, cryoglobulinemia, right-sided endocarditis, lyphomatoid granulomatosis, sarcoidosis, scleroderma
- Toxins—ingestion of paraquat or diquat
- Infections—Legionnaires' disease, *Mycoplasma* infection, tuberculosis, disseminated viral or fungal infection

AKI from any cause with hypervolemia and pulmonary edema

Lung cancer with hypercalcemia, tumor lysis, or glomerulonephritis

AKI and Liver Disease

Prerenal azotemia
- Reduced true (gastrointestinal [GI] hemorrhage, GI losses from lactulose, diuretics, large-volume paracentesis) circulatory volume or effective (hypoalbuminemia, splanchnic vasodilation)
- Hepatorenal syndrome type 1 or 2
- Tense ascites with abdominal compartment syndrome

Intrinsic AKI
- Ischemic (severe hypoperfusion; see previously) or direct nephrotoxicity and hepatotoxicity of drugs or toxins (e.g., carbon tetrachloride, acetaminophen, tetracyclines, methoxyflurane)
- Tubulointerstitial nephritis + hepatitis caused by drugs (e.g., sulfonamides, rifampin, phenytoin, allopurinol, phenindione), infections (leptospirosis, brucellosis, Epstein-Barr virus, cytomegalovirus), malignant infiltration (leukemia, lymphoma) or sarcoidosis
- Glomerulonephritis or vasculitis (e.g., polyarteritis nodosa, ANCA–associated GN, cryoglobulinemia, systemic lupus erythematosus, postinfectious hepatitis or liver abscess

AKI and Nephrotic Syndrome

Prerenal azotemia
- Intravascular volume depletion (diuretic therapy, hypoalbuminemia)

Intrinsic AKI
- Manifestation of primary glomerular disease
- Collapsing glomerulopathy (e.g., HIV, pamidronate)
- Associated ATN (older hypertensive men)
- Associated interstitial nephritis (NSAIDs, rifampin, interferon alfa)

Other—amyloid or light chain deposition disease, renal vein thrombosis, severe interstitial edema

AKI, Acute kidney injury; *ANCA,* antineutrophil cytoplasmic antibody; *ATN,* acute tubular necrosis; *GN,* glomerulonephritis; *HELLP,* ***h***emolysis, ***e***levated ***l***iver enzymes, ***l***ow ***p***latelets; *NSAIDs,* nonsteroidal antiinflammatory drugs; *SLE,* systemic lupus erythematosus.

of dialysis membranes that are permeable to light chains and other proteins with molecular weights lower than albumin (high-cutoff membranes) has also been proposed as a potential therapeutic strategy; however, data from clinical trials evaluating the efficacy of this strategy are similarly conflicting.[173–175]

ACUTE KIDNEY INJURY IN PREGNANCY

In the industrialized world, the incidence of dialysis-requiring AKI in the setting of pregnancy is approximately 1 in 20,000 births.[176,177] The marked decline in this complication over the past 50 years is a result of improved prenatal care and

Box 29.5 Cairo-Bishop Definition of Tumor Lysis Syndrome

Diagnosis of Laboratory Tumor Lysis Syndrome

Requires at least two of the following criteria achieved in the same 24-hour interval from 3 days before to 7 days after chemotherapy initiation:

- Uric acid ≥ 8.0 mg/dL or ≥25% increase from baseline
- Potassium ≥ 6.0 mmol/L or ≥25% increase from baseline
- Phosphorus ≥ 4.6 mg/dL (≥6.5 mg/dL in children) or ≥25% increase from baseline
- Calcium ≤ 7.0 mg/dL or ≥25% decrease from baseline

Diagnosis of Clinical Tumor Lysis Syndrome

Laboratory tumor lysis syndrome plus at least one of the following:

- Serum creatinine ≥1.5 times the age-adjusted upper limit of normal
- Cardiac arrhythmia/sudden death
- Seizure

Modified from Cairo MS, Bishop M. Tumour lysis syndrome: new therapeutic strategies and classification. Br J Haematol. 2004;127:3–11.

advancements in obstetrics practice. In early pregnancy, ATN induced by nephrotoxic abortifacients remains a relatively common cause of AKI in developing countries, but is rare in the developed world. Ischemic ATN, severe toxemia of pregnancy, and postpartum HUS and TTP are the most common causes of AKI in later term pregnancy.[176,178,179] Ischemic ATN is usually precipitated by placental abruption or postpartum hemorrhage and, less commonly, by amniotic fluid embolism or sepsis. Glomerular filtration is usually normal in mild or moderate preeclampsia; however, AKI may complicate severe preeclampsia.[179,180] In this setting, AKI is typically transient and found in association with intrarenal vasospasm, marked hypertension, and neurologic abnormalities.

A variant of preeclampsia, the HELLP syndrome (***h***emolysis, ***e***levated ***l***iver enzymes, ***l***ow ***p***latelets), is characterized by a benign initial course that can rapidly deteriorate with the development of thrombotic microangiopathy with hemolysis, coagulation abnormalities, derangement in hepatic function, and AKI.[180–182] Immediate delivery of the fetus is indicated in this setting. Thrombotic microangiopathy can also develop in the postpartum setting and typically occurs in patients who have had a normal pregnancy.[183] Postpartum thrombotic microangiopathy is characterized by thrombocytopenia, microangiopathic anemia, and normal prothrombin and partial thromboplastin times and frequently results in long-term impairment of renal function.

Acute fatty liver of pregnancy (AFLP) occurs in approximately 1 in 7000 pregnancies and is associated with AKI, likely as a result of intrarenal vasoconstriction, as occurs in the hepatorenal syndrome. Although the exact origin of AFLP is unknown, the incidence is increased in women who carry a fetus with a defect in fatty acid oxidation and who are themselves carriers of a genetic mutation that compromises intramitochondrial fatty acid oxidation.[182] Acute bilateral pyelonephritis may also precipitate AKI in pregnancy and should be obvious from the patient's presentation (fever, flank pain), findings on urinalysis (bacteria, leukocytes), and laboratory tests (leukocytosis, elevated serum creatinine level).[178,181,184,185] The diagnosis of postrenal AKI in the pregnant patient is particularly challenging due to the physiologic dilation of the collecting system that normally occurs in the second and third trimesters. As a result, determining the presence of abnormal findings on renal ultrasound is more difficult.

ACUTE KIDNEY INJURY IN THE SETTING OF CARDIAC SURGERY

An acute deterioration in kidney function is a relatively common complication following cardiac surgery, with an incidence of 7.7% to 42%, depending on the criteria used to define AKI.[186–190] AKI requiring dialytic support occurs in up to 5% of patients following cardiac surgery.[186–190] AKI in the perioperative period is usually attributed to prerenal azotemia associated with decreased cardiac function or to ATN. Risk factors for cardiac surgery–associated AKI can be broadly categorized into presurgical patient-related factors, surgical factors, and postoperative events. The principal patient-related risk factors include underlying CKD, advanced age, left ventricular dysfunction, previous myocardial revascularization, diabetes mellitus, and peripheral vascular disease.[189–192] Operative factors include the need for emergent surgery, prolonged time on cardiopulmonary bypass, insertion of an intraaortic balloon pump, performance of concomitant valvular surgery, and redo coronary artery bypass grafting (CABG). Several studies have compared the incidence of AKI following on-pump versus off-pump CABG, with some data suggesting that off-pump CABG is associated with a lower incidence of AKI.[193–197] Postoperative factors associated with an increased risk for AKI include reduced cardiac output, bleeding, vasodilatory shock, and the overzealous use of diuretics and afterload reducing agents.

Additional potential causes of AKI following CABG include the administration of iodinated contrast media in the pre-, peri-, and/or postoperative period, antibiotic-associated acute interstitial nephritis, and atheroembolic disease.[198] Whereas prerenal azotemia and ATN typically occur within days of the surgical procedure, atheroembolic AKI may take longer to develop and can be distinguished by the characteristic clinical features of livedo reticularis, cyanosis, and gangrenous digital lesions, as well as the findings of eosinophilia, eosinophiluria, and hypocomplementemia.

ACUTE KIDNEY INJURY AFTER SOLID ORGAN OR BONE MARROW TRANSPLANTATION

Nonrenal solid organ transplant recipients have a particularly high risk of AKI from cardiopulmonary and hepatic failure, sepsis, and the nephrotoxic effects of antimicrobial and immunosuppressive agents. In a large retrospective multicenter study, 25% of all nonrenal solid organ transplant recipients developed AKI, with 8% requiring RRT.[199] The development of AKI requiring dialysis was associated with a 9- to 12-fold increase in mortality. AKI developed in 35% of heart transplant recipients and in 15% of lung transplant

recipients. As many as 30% of liver transplant recipients develop AKI, many of whom had CKD prior to transplantation.[200,201] There are conflicting data as to whether impaired kidney function pretransplantation predicts outcomes in patients undergoing orthotopic liver transplantation; however, patients with impaired kidney function preoperatively have longer hospital and ICU stays and are more likely to need dialysis compared with patients with intact preoperative kidney function.[202–204]

AKI is a well-recognized complication of hematopoietic cell transplantation.[144,205,206] The three types of hematopoietic cell transplantation are myeloablative autologous, myeloablative allogeneic, and nonmyeloablative allogeneic, and the incidence, severity, and outcomes of AKI following these forms of hematopoietic cell transplantation vary considerably.[205,207,208] In a study of 272 patients who underwent myeloablative hematopoietic cell transplantation (predominantly allogeneic), 53% developed AKI and 24% required dialysis.[209] Of patients with dialysis requiring AKI, the mortality rate was 84%. One study has found an incidence of severe AKI in this patient population of 73%.[210]

AKI following nonmyeloablative allogeneic hematopoietic cell transplantation is less common.[210,211] A study of 253 patients demonstrated an incidence of AKI of 40% within 3 months of hematopoietic cell transplantation, with just 4.4% of patients requiring dialysis.[211] The incidence of AKI following myeloablative autologous hematopoietic cell transplantation is considerably lower.[212,213] A study of 173 patients following autologous hematopoietic cell transplantation reported an incidence of AKI of 21%, with 5% of patients requiring dialysis.[213] The absence of graft-versus-host disease and more rapid engraftment likely account for the lower incidence of AKI in this setting. Causes of hematopoietic cell transplantation-associated AKI include hypovolemia, sepsis, tumor lysis syndrome, direct tubular toxicity from cytoreductive therapy, thrombotic microangiopathy, graft-versus-host disease, antibiotics, immunosuppressive agents, and hepatic venoocclusive disease (VOD).

VOD results from acute radiochemotherapy-induced endothelial cell injury of hepatic venules.[209,214–216] This condition usually occurs in conditioning regimens that include total body irradiation and cyclophosphamide and/or busulfan and in the setting of myeloablative allogeneic hematopoietic cell transplantation. The syndrome is characterized clinically by profound jaundice and avid salt retention, with edema and ascites within the first month after engraftment and the subsequent development of AKI. Oliguric AKI is common in moderate VOD and certain in severe cases. The mortality rate for patients with severe VOD approaches 100%.

BK virus is a human polyoma virus that is a common opportunistic infection in solid organ transplant recipients and in patients following hematopoietic cell transplantation.[217] Detectable BK viruria may be seen in as many as 50% of patients undergoing hematopoietic cell transplantation.[218] Reactivation of latent BK virus infection in immunosuppressed patients is associated with both hemorrhagic cystitis and renal involvement with tubular atrophy and fibrosis, with an inflammatory lymphocytic infiltrate with intranuclear BK virus inclusion bodies.[219] The diagnosis is suggested by rising viral titers in blood and/or urine; the mainstay of treatment is minimization of immunosuppression.

ACUTE KIDNEY INJURY ASSOCIATED WITH PULMONARY DISEASE

The coexistence of AKI and pulmonary disease (pulmonary-renal syndrome) typically suggests the presence of Goodpasture syndrome, antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis, or other vasculitides.[220–222] The detection of antiglomerular basement membrane antibodies, antineutrophil cytoplasmic antibodies, or low serum complement concentrations can be helpful in differentiating among the various causes of pulmonary-renal syndrome, although the urgent need for definitive diagnosis and treatment may mandate lung or renal biopsy. Several toxic ingestions and infections may also precipitate simultaneous pulmonary and kidney injury that mimics vasculitis-associated pulmonary-renal syndrome. Furthermore, AKI of any cause may be complicated by secondary hypervolemia and pulmonary edema. Severe lung disease and ventilator support with increased intrathoracic pressure may compromise cardiac output and induce prerenal AKI.

ACUTE KIDNEY INJURY IN ASSOCIATION WITH LIVER DISEASE

The differential diagnosis of AKI in patients with liver disease is broad. Common causes of AKI in this setting include intravascular volume depletion, gastrointestinal bleeding, sepsis, and nephrotoxins. Most cases of AKI in advanced liver disease are due to prerenal azotemia, ATN, or HRS, and differentiating these conditions can be clinically challenging.[223–225] Although a urine sodium concentration less than 20 mmol/L and fractional excretion rate of sodium less than 1% are typical of prerenal AKI and HRS, high-dose diuretics, which are commonly prescribed in patients with advanced liver disease, may lead to higher sodium excretion rates. Differentiating ATN from other forms of AKI is further confounded by the fact that bile-stained casts, which can be seen in prerenal AKI and HRS, have a similar appearance to the classic, muddy brown, granular casts of ATN.[226] Kidney disease in patients with liver disease may also result from acute glomerular disease, including immunoglobulin A (IgA) nephropathy, hepatitis B virus–associated membranous nephropathy, and hepatitis C virus–associated membranoproliferative glomerulonephritis with cryoglobulinemia. Acetaminophen toxicity may cause nephrotoxic ATN in addition to being one of the most common causes of acute hepatotoxicity.

The term *hepatorenal syndrome* is typically reserved for a clinical syndrome marked by irreversible AKI that develops in patients with advanced cirrhosis, although it has also been described in the setting of fulminant viral and alcoholic hepatitis. HRS almost certainly represents the terminal stage of a state of hypoperfusion that begins early in the course of chronic liver disease. The precise pathophysiologic mechanisms underlying the hemodynamic alterations in HRS are incompletely understood. In the early stages of HRS, increased vascular capacitance as the result of splanchnic and systemic vasodilation is thought to trigger activation of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system.[223] Renal perfusion is preserved in this stage by the local release of renal vasodilatory factors; however, these compensatory mechanisms are eventually overwhelmed, and progressive renal hypoperfusion ensues.

An inadequate increase in cardiac output relative to the fall in vascular resistance is also thought to contribute to the development of HRS.

Clinically, the presentation of HRS closely resembles that of prerenal AKI. However, unlike prerenal AKI, HRS does not improve with aggressive expansion of the intravascular space. Criteria for the diagnosis of HRS have undergone revision (Box 29.6).[227] Previous criteria were based on an increase in the serum creatinine level to more than 1.5 mg/dL in the setting of cirrhosis with ascites,[228] whereas the updated criteria have been harmonized with the creatinine component of the KDIGO AKI definition.[227] Other criteria include failure of kidney function to improve after at least 2 days with diuretic withdrawal and volume expansion with albumin, the absence of shock or concurrent or recent treatment with nephrotoxic drugs, and the absence parenchymal kidney disease—defined by proteinuria more than 500 mg/day, hematuria (>50 RBC/high power field [hpf]), and/or abnormal renal ultrasonography.[227]

Two subtypes of HRS have been described. Under the prior criteria, type 1 HRS was characterized by a rapid onset of AKI defined by at least a doubling of the serum creatinine concentration to a level of at least 2.5 mg/dL or a reduction in glomerular filtration of 50% or more to a level less than 20 mL/min over a 2-week period.[229,230] In the revised criteria, the diagnosis of type 1 HRS is based on meeting KDIGO criteria for stage 2 or higher AKI (i.e., doubling of serum creatinine level from baseline).[227] Type 1 HRS typically develops in hospitalized patients and may be precipitated by variceal bleeding, overly rapid diuresis, the performance of paracentesis or, most commonly, the development of spontaneous bacterial peritonitis. Other postulated triggers include infections, minor surgery, or the use of NSAIDs or other drugs. However, caution must be exerted in these cases to exclude reversible causes of AKI. Type 1 HRS is generally characterized by a fulminant course with oliguria, encephalopathy, marked hyperbilirubinemia, and death within 1 month of the clinical presentation. However, advances in the management of HRS (discussed later) have suggested that there may be a trend toward better survival in patients who respond to therapy.[231,232] Type 2 HRS is typified by a more gradual decline in renal function that develops in the setting of diuretic resistant ascites and avid sodium retention. The prognosis of type 2 HRS is considerably better than that of type 1 HRS, with a reported median survival of 6 months and a 1-year survival rate as high as 30%.[233,234] The development of a sudden deterioration in kidney function after a prolonged stable period may occur in patients with type 2 HRS, leading to outcomes similar to those of patients with type 1 HRS.

Definitive treatment of HRS is dependent on the recovery of hepatic function or successful liver transplantation. However, the use of vasoconstrictive agents combined with volume expansion with colloid has shown promise for improving kidney function.[235–237] It is postulated that by reversing the splanchnic and peripheral vasodilation, more normal renal perfusion can be restored. Vasoconstrictive regimens that have been used include norepinephrine, combination therapy with midodrine and octreotide, and the vasopressin agonist terlipressin.[235–241] Although vasoconstrictive therapy is associated with improvement in kidney function, and patients who respond have an improved prognosis, the use of vasoconstrictive therapy has not been shown to improve overall prognosis in patients with AKI, suggesting that survival remains limited by the underlying severity of the liver disease.

Box 29.6 Diagnostic Criteria for Hepatorenal Syndrome

- Cirrhosis with ascites
- Acute kidney injury (AKI) defined as:
  - ≥0.3-mg/dL increase in serum creatinine over <48 hours
  - ≥50% increase in serum creatinine level known or presumed to have occurred within prior 7 days
- No improvement of serum creatinine level (decrease to a level of ≤1.5 mg/dL) after at least 2 days of diuretic withdrawal and volume expansion with albumin (1 g/kg body weight per day to a maximum of 100 g/day)
- Absence of shock
- Absence of parenchymal kidney disease as indicated by:
  - Proteinuria >500 mg/day
  - Microhematuria (>50 red blood cells/high power field); and/or
  - Abnormal renal ultrasonography

Type 1 Hepatorenal Syndrome

Rapid progressive AKI defined based on increase in serum creatinine level by >2× baseline

Type 2 Hepatorenal Syndrome

Moderate renal dysfunction with a steady or slowly progressive course

Modified from Angeli P, Gines P, Wong F, et al. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. Gut. 2015;64:531–537.

ACUTE KIDNEY INJURY AND THE NEPHROTIC SYNDROME

AKI in the context of the nephrotic syndrome presents a unique array of potential diagnoses. Epithelial injury, if severe, can trigger both nephrotic range proteinuria and acute or subacute kidney injury.[242,243] The epithelial injury typically occurs as a manifestation of primary glomerular disease, such as collapsing glomerulopathy or crescentic membranous nephropathy. Less dramatic visceral epithelial cell injury, in combination with proximal tubular injury (e.g., panepithelial cell injury induced by NSAIDs or possible undiagnosed viral illness) or interstitial nephritis (e.g., rifampicin- or ampicillin-induced) can also present as AKI complicating the nephrotic syndrome.[244–246] Massive excretion of light chain proteins in patients with multiple myeloma may also present in this fashion.[247,248] ATN in association with nephrotic syndrome is seen in a subpopulation of older patients with minimal change disease, and in other patients with nephrosis and severe hypoalbuminemia, particularly with overzealous diuresis. In general, patients with AKI complicating the nephrotic syndrome have higher blood pressure and urinary protein excretion than patients without AKI.[242] The higher incidence of arteriosclerosis in biopsy samples from these

patients may point to preexisting hypertensive nephrosclerosis as a risk factor for the development of this complication. Renal vein thrombosis must always be considered in the differential diagnosis of the nephrotic syndrome and AKI, particularly in the pediatric population and in adults with membranous nephropathy in association with high-grade proteinuria and hypoalbuminemia.

COMPLICATIONS OF ACUTE KIDNEY INJURY

The acute loss of kidney function in AKI results in multiple derangements in fluid, electrolyte, and acid-base homeostasis and in hematologic, gastroenterologic, and immunologic function (Table 29.6).

POTASSIUM HOMEOSTASIS

Hyperkalemia is a common and potentially life-threatening complication of AKI.[249,250] The serum K^+ level typically rises by 0.5 mmol/L/day in oligoanuric patients and reflects impaired excretion of K^+ derived from a patient's diet, the administration of K^+-containing solutions and drugs administered as potassium salts, and the release of K^+ from the injured tubular epithelium. Hyperkalemia may be compounded by coexistent metabolic acidosis and/or hyperglycemia or other hyperosmolar states that promote K^+ efflux from cells. Hyperkalemia present at the time of diagnosis of AKI or the rapid development of severe hyperkalemia suggests massive tissue destruction, as might be seen with rhabdomyolysis, hemolysis, or tumor lysis.[50,90,251] Hyperuricemia and hyperphosphatemia may accompany hyperkalemia in these settings. Mild hyperkalemia (<6.0 mmol/L) is usually asymptomatic. Higher levels are frequently associated with electrocardiographic abnormalities, including, peaked T waves, prolongation of the PR interval, flattening of P waves, widening of the QRS complex, and intraventricular conduction defects.[252–254] These electrocardiographic findings may precede the onset of life-threatening cardiac arrhythmias, such as bradycardia, heart block, ventricular tachycardia, ventricular fibrillation, and asystole. In addition, hyperkalemia may induce neuromuscular abnormalities, such as paresthesias, hyporeflexia, weakness, ascending flaccid paralysis, and respiratory failure.

Hypokalemia is unusual in AKI but may complicate nonoliguric ATN caused by aminoglycosides, cisplatin, or amphotericin B, presumably because of impaired K^+ reabsorption resulting from epithelial cell injury in the thick ascending limb of the loop of Henle.[255,256]

ACID-BASE HOMEOSTASIS

Normal metabolism of dietary protein yields between 50 and 100 mmol/day of fixed nonvolatile acids (principally sulfuric and phosphoric acids) that are excreted by the kidneys to maintain acid-base homeostasis. Predictably, AKI is commonly complicated by metabolic acidosis, typically with a widening of the serum anion gap due to retention of phosphates, sulfates, and organic anions.[257] Acidosis may be severe (daily fall in plasma HCO_3^- >2 mmol/L) when the generation of H^+ is increased by additional mechanisms (e.g., diabetic or fasting ketoacidosis, lactic acidosis complicating generalized tissue hypoperfusion, liver disease, or sepsis, and metabolism of ethylene glycol).[70,225,258] In contrast, metabolic alkalosis is an infrequent finding, but may complicate overly aggressive correction of acidosis with HCO_3^-, overzealous use of combination loop and thiazide diuretics, or loss of gastric acid by vomiting or nasogastric aspiration.

MINERAL AND URIC ACID HOMEOSTASIS

Mild to moderate hyperphosphatemia (5–10 mg/dL) is a common consequence of AKI, and hyperphosphatemia may be severe (10–20 mg/dL) in highly catabolic patients or when AKI is associated with rapid cell death as in rhabdomyolysis, severe burns, hemolysis, or tumor lysis.[259–262] Factors that potentially contribute to hypocalcemia include skeletal resistance to the actions of parathyroid hormone, reduced levels of 1,25-dihydroxyvitamin D, Ca^{2+} sequestration in injured tissues, such as muscle in the setting of rhabdomyolysis, and metastatic deposition of calcium phosphate salts in the setting of severe hyperphosphatemia.[263–265]

Table 29.6 Common Complications of Acute Kidney Injury

Metabolic	Cardiovascular	Gastrointestinal	Neurologic	Hematologic	Infectious	Other
Hyperkalemia	Pulmonary edema	Nausea	Neuromuscular irritability	Anemia	Pneumonia	Hiccups
Metabolic acidosis	Arrhythmias	Vomiting	Asterexis	Bleeding	Septicemia	Elevated parathyroid hormone level
Hyponatremia	Pericarditis	Malnutrition	Seizures		Urinary tract infection	Low total triiodothyronine and thyroxine
Hypocalcemia	Pericardial effusion	Hemorrhage	Mental status changes			Normal thyroxine level
Hyperphosphatemia	Pulmonary embolism					
Hypermagnesemia	Hypertension					
Hyperuricemia	Myocardial infarction					

Hypocalcemia is usually asymptomatic, possibly because of the counterbalancing effects of acidosis on neuromuscular excitability. However, symptomatic hypocalcemia can occur in patients with rhabdomyolysis or acute pancreatitis or after treatment of acidosis with HCO_3^-.[263] Clinical manifestations of hypocalcemia include perioral paresthesias, muscle cramps, seizures, hallucinations, and confusion, as well as prolongation of the QT interval and nonspecific T wave changes on the electrocardiogram (ECG). The Chvostek sign (contraction of facial muscles on tapping of the jaw over the facial nerve) and Trousseau sign (carpopedal spasm after occlusion of arterial blood supply to the arm for 3 minutes with a blood pressure cuff) are useful indicators of latent tetany in high-risk patients.

Mild asymptomatic hypermagnesemia is common in oliguric AKI and reflects the impaired excretion of ingested magnesium—dietary magnesium, magnesium-containing laxatives, or antacids.[266,267] More significant hypermagnesemia is usually the result of overzealous parenteral magnesium administration, as in the management of AKI associated with preeclampsia. Hypomagnesemia occasionally complicates nonoliguric ATN associated with cisplatin or amphotericin B and, as with hypokalemia, likely reflects injury to the thick ascending limb of loop of Henle, a principal site for Mg^{2+} reabsorption.[256,268,269] Hypomagnesemia is usually asymptomatic but may occasionally manifest as neuromuscular instability, cramps, seizures, cardiac arrhythmias, or resistant hypokalemia or hypocalcemia.[266,270]

Uric acid is cleared from blood by glomerular filtration and secretion by proximal tubule cells, and asymptomatic hyperuricemia (12–15 mg/dL) is typical in established AKI. Higher levels suggest increased production of uric acid and may point to a diagnosis of acute urate nephropathy.[271–273] The urinary uric acid-to-creatinine ratio on a random specimen has been proposed as a means to distinguish between hyperuricemia caused by overproduction and impaired excretion. In a small series of patients, this ratio was more than 1 in 5 patients with acute uric acid nephropathy and was less than 1 in 27 patients with acute kidney injury due to other causes.[274] In a subsequent case series, elevations in the uric acid-to-creatinine ratio to values of more than 1 were described in other etiologies of AKI, most notably patients with infections who were markedly hypercatabolic.[275]

VOLUME OVERLOAD AND CARDIAC COMPLICATIONS

Extracellular volume overload is an almost inevitable consequence of diminished salt and water excretion in AKI and may present clinically as mild hypertension, increased jugular venous pressure, pulmonary vascular congestion, pleural effusion, ascites, peripheral edema, increased body weight, and life-threatening pulmonary edema. Hypervolemia may be particularly troublesome in patients receiving multiple IV medications, high volumes of enteral or parenteral nutrition, and/or excessive volumes of maintenance IV fluids. Moderate or severe hypertension is unusual in ATN and should suggest other diagnoses, such as hypertensive nephrosclerosis, glomerulonephritis, preeclampsia, renal artery stenosis, and other diseases of the renal vasculature.[178,276–278] Excessive water ingestion or the administration of a hypotonic saline or dextrose solution can trigger hyponatremia, which, if severe, may cause cerebral edema, seizures, and other neurologic abnormalities.[279] Cardiac complications include arrhythmias and myocardial infarction. Although these events may reflect primary cardiac disease, abnormalities in myocardial contractility and excitability may be triggered or compounded by hypervolemia, acidosis, hyperkalemia, and other metabolic sequelae of AKI.[280]

HEMATOLOGIC COMPLICATIONS

Anemia develops rapidly in AKI and is usually multifactorial in origin. Contributing factors include inhibition of erythropoiesis, hemolysis, bleeding, hemodilution, and reduced RBC survival time.[281–283] Prolongation of the bleeding time is also common, resulting from mild thrombocytopenia, platelet dysfunction, and clotting factor abnormalities (e.g., factor VIII dysfunction).

NUTRITIONAL AND GASTROINTESTINAL COMPLICATIONS

Malnutrition remains one of the most frustrating and troublesome complications of AKI. Most patients have net protein breakdown, which may exceed 200 g/day in catabolic patients.[284–286] Malnutrition is usually multifactorial in origin and may reflect an inability to eat, loss of appetite, and/or inadequate nutritional support, the catabolic nature of the underlying medical disorder (e.g., sepsis, rhabdomyolysis, trauma), nutrient losses in drainage fluids or dialysate, and increased breakdown and reduced synthesis of muscle protein and increased hepatic gluconeogenesis, probably through the actions of toxins, hormones (e.g., glucagon, parathyroid hormone), or other substances (e.g., proteases) that accumulate in AKI.[287–291] Nutrition may also be compromised by the high incidence of acute gastrointestinal hemorrhage, which complicates up to 15% of cases of AKI. Mild gastrointestinal bleeding is common (10%–30%) and is usually due to stress ulceration of gastric or small intestinal mucosa.[292,293]

INFECTIOUS COMPLICATIONS

Infection is the most common and serious complication of AKI, occurring in 50% to 90% of cases and accounting for up to 75% of deaths.[25,249,294–296] It is unclear whether this high incidence of infection is due to a defect in host immune responses or to repeated breaches of mucocutaneous barriers (e.g., IV cannulas, mechanical ventilation, bladder catheterization) resulting from therapeutic interventions.

OTHER SEQUELAE OF ACUTE KIDNEY INJURY

Protracted periods of severe AKI or short intervals of catabolic anuric AKI often lead to the development of the uremic syndrome. Clinical manifestations of the uremic syndrome, in addition to those already listed, include pericarditis, pericardial effusion, and cardiac tamponade; gastrointestinal complications such as anorexia, nausea, vomiting, and ileus; and neuropsychiatric disturbances, including lethargy, confusion, stupor, coma, agitation, psychosis, asterixis, myoclonus, hyperreflexia, restless legs syndrome, focal neurologic deficit, and/or seizures. The uremic toxin(s) responsible for this

syndrome has (have) yet to be defined. Candidate molecules include urea, other products of nitrogen metabolism such as guanidine compounds, products of bacterial metabolism such as aromatic amines and indoles, and other compounds that are inappropriately retained in the circulation in AKI or are underproduced, such as nitric oxide (NO).[297]

COMPLICATIONS DURING RECOVERY FROM ACUTE KIDNEY INJURY

A vigorous diuresis may complicate the recovery phase of AKI and may precipitate intravascular volume depletion and can result in delayed recovery of kidney function. This diuretic response probably reflects the combined effects of an osmotic diuresis induced by retained urea and other byproducts of protein metabolism, excretion of retained salt and water accumulated during AKI, and delayed recovery of tubular reabsorptive function relative to glomerular filtration leading to salt wasting.[298–301] Hypernatremia may also complicate this recovery phase if free water losses are not replenished or are inappropriately replaced by relatively hypertonic saline solutions. Hypokalemia, hypomagnesemia, hypophosphatemia, and hypocalcemia are rarer metabolic complications during recovery from AKI. Mild transient hypercalcemia is relatively frequent during recovery and appears to be a consequence of delayed resolution of secondary hyperparathyroidism. In addition, hypercalcemia may complicate recovery from rhabdomyolysis because of mobilization of sequestered Ca^{2+} from injured muscle.[302]

MANAGEMENT OF ACUTE KIDNEY INJURY

The treatment of AKI varies considerably, based on its cause and clinical presentation. Evidence-based pharmacologic therapy to counteract pathophysiologic processes in the kidney and arrest renal parenchymal damage is not available for certain forms of AKI, notably ATN. In such cases, the management of AKI focuses on implementing interventions to prevent its development, when possible, providing supportive care to ameliorate derangements of fluid and electrolyte homeostasis, and instituting treatment to prevent and mitigate uremic complications (Table 29.7). In cases of severe AKI, RRT is often required. The ultimate goals of management are to prevent death, facilitate recovery of kidney function, and minimize the risk for de novo and/or progressive CKD.

MANAGEMENT OF PRERENAL ACUTE KIDNEY INJURY

INTRAVASCULAR VOLUME DEPLETION

Prerenal AKI is defined as hemodynamically mediated kidney dysfunction that is rapidly reversible following normalization of renal perfusion.[303] Prevention of prerenal AKI from intravascular volume depletion involves the early recognition and treatment of conditions that involve the loss of extracellular fluid, including vomiting, diarrhea, excessive diuresis, and bleeding before underperfusion

Table 29.7 Supportive Management of Acute Kidney Injury

Management Issue	Treatment
Intravascular volume overload	Restriction of salt (<1–2 g/day) and water (<1 L/day) intake
	Diuretic therapy (if nonoliguric)
	Ultrafiltration
Hyponatremia	Restriction of oral and intravenous free water
Hyperkalemia	Calcium gluconate (10 mL of 10% solution over 5 min) if ECG changes present
	Insulin (10–20 Units) IV push + glucose (250 mL of 20%) IV over 30–60 min
	Albuterol (10–20 mg by nebulizer or MDI)
	Renal replacement therapy
	Loop diuretics (if nonoliguric)
	K^+ binding resin
	Discontinue K^+ supplements or K^+-sparing diuretics
	Restriction of dietary potassium
Metabolic acidosis	Restriction of dietary protein
	Sodium bicarbonate (if HCO_3^- <15 mmol/L)
	Renal replacement therapy
Hyperphosphatemia	Restriction of dietary phosphate intake
	Phosphate binding agents (aluminum hydroxide, calcium carbonate, calcium acetate, sevelamer, lanthanum)
Hypocalcemia	Oral or intravenous replacement (if symptomatic or sodium bicarbonate to be administered)
Hypermagnesemia	Discontinue magnesium containing antacids
Nutrition	Caloric intake: 20–30 kcal/day
	Protein intake:
	Nondialysis-requiring—0.8–1.0 g/kg/day
	Dialysis-requiring—1.0–1.5 g/kg/day
	Continuous renal replacement therapy—up to 1.7 g/kg/day
	Enteral route of nutrition preferred
Drug dosage	Adjust all doses for GFR and renal replacement modality

ECG, Electrocardiographic; *GFR,* glomerular filtration rate; *MDI,* metered-dose inhaler.

of the kidneys occurs. In patients in whom intravascular volume depletion leads to prerenal AKI, treatment consists of restoration of a normal circulating blood volume. The optimal composition of administered fluids in patients with hypovolemic prerenal AKI depends on the source of fluid loss and associated electrolyte and acid-base disturbances. The initial management commonly consists of intravascular volume resuscitation with an isotonic crystalloid solution. Recent studies have demonstrated that balanced crystalloids reduce major adverse kidney events compared with isotonic saline in hospitalized patients.[304,305] Red blood cell transfusion should be used for hemorrhagic hypovolemia when there is ongoing bleeding, particularly if the patient is hemodynamically unstable, or if the blood hemoglobin concentration is dangerously low.

The relative merits of colloid and crystalloid resuscitation fluids in the management of nonhemorrhagic renal, extrarenal, and third space fluid losses are controversial, with advocates for the use of colloids positing that they are more effective at restoring circulating blood volume due to greater retention in the intravascular compartment. However, randomized controlled trials (RCTs) and meta-analyses comparing colloid with crystalloid replacement for resuscitation in critically ill patients have not confirmed this theoretic benefit and demonstrated an increased need for RRT, and other adverse outcomes were associated with colloid formulations containing hydroxyethyl starch.[306–313] In a meta-analysis of 55 trials involving 3504 patients randomly assigned to treatment with albumin or crystalloid, there was no evidence of improved outcomes, decreased mortality, or other complications associated with albumin administration.[314] These results were subsequently confirmed in a nearly 7000-patient multicenter RCT of fluid resuscitation in hypovolemic medical and surgical ICU patients, in which 28-day survival, development of single or multiple organ failure, and duration of hospitalization were similar in both groups.[315] Although specific data on the development of AKI were not described, the need for RRT was similar with saline compared with albumin resuscitation. However, in a post hoc analysis of patients with traumatic brain injury, albumin resuscitation was associated with increased mortality risk.[316]

The use of synthetic colloid solutions has been proposed as an alternative to albumin administration; however, hydroxyethyl starch preparations have been associated with an increased risk of AKI. In a multicenter RCT comparing fluid resuscitation with hydroxyethyl starch with a 3% gelatin solution in 129 patients with sepsis, hydroxyethyl starch was associated with a more than twofold increased risk of AKI.[309] A subsequent meta-analysis has confirmed the increased risk of AKI associated with hydroxyethyl starch across 34 studies that included 2604 individuals.[310] In an ensuing RCT that included 7000 critically ill patients who were assigned to receive 6% hydroxyethyl starch or isotonic saline, there was an approximately 20% increased risk of AKI treated with RRT with the use of hydroxyethyl starch.[313] Based on these data demonstrating no benefit and potential increased risk of AKI, along with the higher costs associated with colloid administration, their routine use for volume resuscitation in hypovolemia and sepsis is not advisable. In particular, hydroxyethyl starch solutions should be used very sparingly and, if used, there should be regular monitoring of kidney function. In such cases, the risk of hyperoncotic renal failure should be minimized by the concomitant use of appropriate crystalloid solutions.[3,309,310,312,313]

Experimental data have suggested that volume resuscitation with isotonic sodium chloride solutions, which contain supraphysiologic concentrations of chloride, may exacerbate renal vasoconstriction and diminish the GFR, as compared with isotonic crystalloid solutions with a lower chloride content.[317–319] In healthy patients, magnetic resonance imaging (MRI) has demonstrated that the infusion of isotonic saline is associated with reduced renal blood flow velocity and renal cortical tissue perfusion as compared with administration of a reduced chloride isotonic crystalloid solution.[320] In a subsequent open-label, sequential period study conducted in a single ICU, replacing the use of high-chloride IV solutions with fluids containing a lower chloride content was associated with a reduction in the incidence of KDIGO stage 3 AKI, from 14% to 8.4%, and in the use of RRT from 10% to 6.3%.[321] A subsequent meta-analysis that included 21 studies, 15 of which were small RCTs, found that the use of high-chloride IV fluid was associated with an increased risk of AKI, with no effect on mortality.[322] However, exclusion of heavily weighted studies in this analysis rendered the association of high-chloride fluid with AKI nonstatistically significant.

Two more recent large pragmatic, cluster-randomized clinical trials conducted in parallel at the same institution compared the administration of isotonic saline with balanced crystalloid solution (i.e., fluid with electrolyte composition that more closely resembles plasma).[304,305] In the Saline Against Lactated Ringer's of Plasma-Lyte in the Emergency Department (SALT-ED) trial of over 13,000 noncritically ill patients, balanced crystalloid was associated with a decrease in the incidence of 30-day major adverse kidney events (i.e., death, new RRT, persistent kidney impairment defined by a >200% increase in serum creatinine level at the time of hospital discharge, within 30 days) compared with saline (4.7% vs. 5.6%; $P = .01$), but not with the primary outcome of hospital-free days (days alive after discharge to day 28).[304] The Isotonic Solutions and Major Adverse Renal Events Trial (SMART), which included 15,802 critically ill patients, found that compared with isotonic saline, balanced crystalloids were associated with a decrease in 30-day major adverse kidney events (14.3% vs. 15.4%; $P = .04$).[305] In the SALT-ED trial, the benefit in the composite outcome was predominantly due to a lower rate of persistent kidney impairment, whereas in SMART, 30-day mortality predominated. Collectively, these findings support a benefit of balanced crystalloid compared with isotonic sodium chloride, yet the benefits were not homogenous. Greatest benefit was present among the critically ill patients with sepsis and among non–critically ill patients who had a baseline serum creatinine level more than 1.5 mg/dL or hyperchloremia (Cl >110 mmol/L) on initial presentation. Although the balanced fluids were more physiologic with regard to their chloride content, they were hypotonic and associated with higher rates of hyponatremia.

The volume and electrolyte content of urinary and gastrointestinal losses, as well as patients' serum electrolyte and acid-base status, should be closely monitored to guide adjustments in the composition of the replacement fluids. Although the potassium content in gastric juices tends to be low, concomitant urinary potassium losses may be quite high as the result of metabolic alkalosis.

HEART FAILURE

The management of AKI in the setting of heart failure is dependent on the clinical setting and cause of the heart failure. In patients with heart failure in whom AKI has developed in the setting of excessive diuresis, withholding diuretics and administering cautious volume replacement may be sufficient to restore kidney function. In acute decompensated heart failure (ADHF), AKI may develop despite worsening volume overload; intensification of diuretic therapy is often required for treatment of pulmonary vascular congestion. Although diuretic therapy may exacerbate prerenal AKI, it can also result in improvement in kidney function via several postulated mechanisms:

1. Decreasing ventricular distention resulting in a shift from the descending limb to the ascending limb of the Starling curve and improvement in myocardial contractility
2. Decreasing venous congestion[318,323–326]
3. Diminishing intraabdominal pressure[327]

Additional therapies for ADHF in the setting of AKI include inotropic support, vasodilators for afterload reduction, and mechanical support, including intraaortic balloon pumps and ventricular assist devices. The use of invasive hemodynamic monitoring in ADHF has been controversial; although it is often used to guide pharmacologic management, clinical data have not demonstrated improved renal outcomes when management is guided by pulmonary artery catheters.[328] The role of isolated ultrafiltration in ADHF is also controversial. Although negative fluid balance can be achieved more readily using extracorporeal ultrafiltration than conventional diuretic therapy, studies have not demonstrated differences in kidney function or survival.[329–331] In the Ultrafiltration Versus Intravenous Diuretics for Patients Hospitalized for Acute Decompensated Heart Failure (UNLOAD) trial, hypervolemic patients with heart failure who were randomized to isolated ultrafiltration had more rapid fluid loss and decreased rehospitalizations within 90 days as compared with patients randomized to diuretic therapy, with no differences in kidney function.[330] In contrast, in the subsequent Cardiorenal Rescue Study in Acute Decompensated Heart Failure (CARRESS-HF) trial, ultrafiltration was inferior to diuretic therapy with respect to the bivariate endpoint of change in serum creatinine level and body weight 96 hours after enrollment ($P = .003$), owing primarily to worsening of kidney function in the ultrafiltration group.[331] Based on these data, extracorporeal ultrafiltration cannot be recommended for the primary management of patients with decompensated heart failure.

LIVER FAILURE AND HEPATORENAL SYNDROME

Although volume-responsive prerenal azotemia is common in patients with advanced liver disease, differentiation from HRS and intrinsic AKI may be difficult.[223] Patients with liver failure are typically total body sodium overloaded with peripheral edema and ascites, but true hypovolemia or reduced effective systemic arterial blood volume is often an important contributory factor to the development AKI. The underlying pathophysiology of salt and water retention in cirrhosis involves multiple pathways. Portal hypertension leads directly to ascites formation, whereas splanchnic and peripheral vasodilation result in a state of relative arterial underfilling, which activates neurohumoral vasoconstrictors that produce intrarenal vasoconstriction, salt and water retention, and decreased GFR.[332] Volume-responsive AKI may develop in the setting of excessive diuresis, increased gastrointestinal losses (often as the result of therapy for hepatic encephalopathy), rapid drainage of ascites, or spontaneous bacterial peritonitis. Worsening hepatic function is often associated with diuretic resistance and progressive or precipitous worsening of kidney function. It has been postulated that an inadequate increase in cardiac output in response to the fall in peripheral vascular resistance may be central to the development of the hepatorenal syndrome.[333]

Differentiation between volume-responsive prerenal AKI and the HRS is based on the clinical response to volume loading. The optimal fluid for volume expansion in this setting has been controversial. Most recent expert opinion has advocated the use of hyperoncotic (20% or 25%) albumin at a dose of 1 g/kg per day.[228,334] However, there is an absence of rigorous data supporting this regimen as compared with volume expansion with isotonic crystalloid solutions. There are more data regarding the use of albumin infusion to prevent AKI in patients undergoing large-volume (>5 L) paracentesis[234,335,336] and in the treatment of spontaneous bacterial peritonitis.[337] In an RCT, patients undergoing paracentesis who received an infusion of approximately 10 g of albumin/L of drained ascites experienced less activation of the RAAS and a significantly lower rate of worsening kidney function than patients who did not receive albumin infusion.[335] In a subsequent study, albumin infusion was superior to the administration of dextran or gelatin solutions in preventing AKI following large-volume paracentesis.[336] Current recommendations are to infuse 6 to 8 g of albumin/L of ascites drained when the paracentesis volume exceeds 5 L. In an RCT comparing antibiotics alone with antibiotics plus albumin for the treatment of spontaneous bacterial peritonitis, infusion of 1.5 g/kg of albumin at the initiation of treatment and an additional 1 g/kg on the third day of treatment was associated with reduced rates of AKI and mortality,[337] although the benefit appears to be restricted to patients in whom the serum creatinine level is more than 1 mg/dL, the blood urea nitrogen is more than 30 mg/dL or the total bilirubin is more than 4 mg/dL.[338]

Definitive therapy of hepatorenal syndrome requires restoration of hepatic function, usually achieved through liver transplantation.[225,334] The role of peritoneovenous shunting (e.g., LeVeen and Denver shunts) in HRS has been inadequately studied. In a subset of 33 patients with HRS included in a randomized trial comparing peritoneovenous shunts to medical therapy, shunting was not associated with improved survival.[339] These data need to be interpreted with caution due to the small sample size and because data on improvement in kidney function were not reported. In addition, as a result of poor long-term patency rates and high rates of complications, particularly encephalopathy, the use of peritoneovenous shunts has largely been supplanted by the use of a transjugular portosystemic shunt (TIPS). TIPS has been demonstrated to provide better control of ascites than sequential paracentesis[340–343] and in one series, lower rates of HRS,[341] albeit with a higher risk of encephalopathy.[344] In a small case series, TIPS was reported to be effective as primary therapy for hepatorenal syndrome,[345] but has not been evaluated in a randomized trial.[334]

Pharmacologic therapy with vasoconstrictors, when combined with albumin infusion, has been associated with improvement in kidney function in patients with hepatorenal syndrome.[334,346] Agents that have shown benefit include norepinephrine,[347] the combination of octreotide and midodrine,[231,348–350] and the V1 vasopressin receptor agonist terlipressin,[235–237,351] although only terlipressin has been evaluated in RCTs. In metaanalyses of published trials, terlipressin was associated with a 3.5- to 4-fold increased odds of reversal of HRS.[352,353] Treatment of HRS with terlipressin was also associated with a modest short-term reduction in mortality; however, longer term outcomes are primarily a function of the underlying liver disease rather than treatment of HRS.[353] In addition, terlipressin was associated with a markedly increased risk of adverse cardiovascular events. More recently, the Reversal of Hepatorenal Syndrome Type 1 with Terlipressin (REVERSE) trial found similar rates of confirmed reversal of HRS with terlipressin and placebo (19.6% vs. 13.1%; $P = .22$), although the mean decrease in serum creatinine level was more pronounced with terlipressin (1.1 mg/dL vs. 0.6 mg/dL; $P = .001$).[237] At present, terlipressin is not approved for use in the United States.

ABDOMINAL COMPARTMENT SYNDROME

Acute kidney injury can result from elevations in intra-abdominal pressure, resulting in a clinical presentation with similar features to prerenal AKI. The abdominal compartment syndrome is defined by an intraabdominal pressure 20 mm Hg or higher associated with dysfunction of one or more organ systems.[354] However, intraabdominal pressures lower than 20 mm Hg may be associated with abdominal compartment syndrome, whereas values higher than this threshold do not universally lead to the abdominal compartment syndrome.[355–358] Abdominal compartment syndrome typically develops in critically ill patients, usually in the setting of trauma with abdominal hemorrhage, abdominal surgery, massive fluid resuscitation, liver transplantation, and gastrointestinal conditions, including peritonitis and pancreatitis. Mechanisms underlying the development of AKI in abdominal compartment syndrome are believed to involve renal vein compression, reduced cardiac output, and renal arterial constriction from sympathetic nervous system and RAAS activation.[359–361] Oliguria, which can lead to anuria, often develops and, as is true for other forms of AKI associated with impaired renal perfusion, urine sodium concentration is commonly reduced.

The diagnosis of abdominal compartment syndrome, which should be suspected in patients with acute abdominal distention and/or rapidly accumulating ascites or abdominal trauma, can be made by simple transduction of the bladder pressure.[59,354,355] Treatment is prompt abdominal decompression; if ascites is present, decompression may be achieved by performing large-volume paracentesis and in patients with severe ileus or colonic distention, bowel decompression may be sufficient; however, surgical laparotomy is often required for definitive therapy.

MANAGEMENT OF POSTRENAL ACUTE KIDNEY INJURY

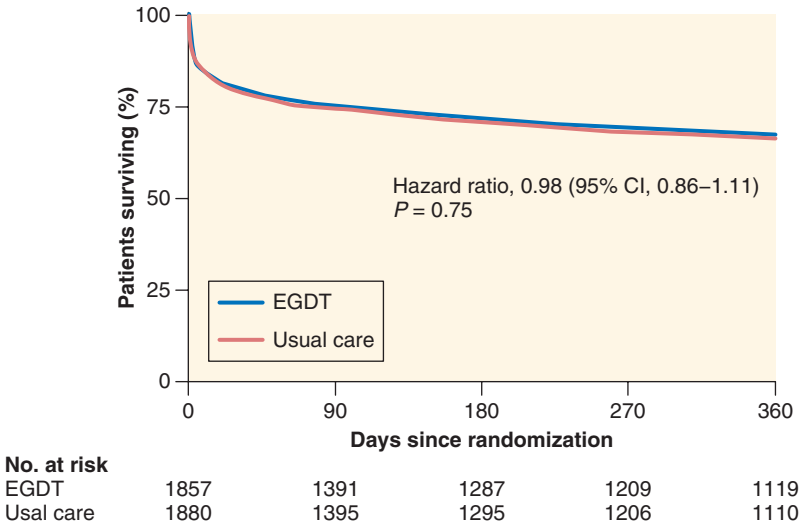
The principle underlying the management of postrenal AKI is the prompt relief of urinary tract obstruction. This topic is reviewed extensively in Chapter 37. Urethral or bladder neck obstruction may be relieved with the placement of a transurethral or suprapubic bladder catheter. Similarly, ureteric obstruction may be acutely relieved by placement of percutaneous nephrostomy tubes or by cystoscopically placed ureteral stents. Following the initial relief of obstruction, most patients experience a physiologic diuresis that resolves after several days as the result of the excretion of volume and solutes retained during the period of renal obstruction. However, approximately 5% of patients may have a more prolonged diuretic phase following relief of obstruction because of delayed recovery of tubule function relative to the GFR, resulting in a salt-wasting syndrome, which may require IV fluid replacement to maintain blood pressure.[300,301,362] Following initial relief of obstruction, urologic evaluation is required for definitive evaluation and management of the underlying cause of obstruction.

PREVENTION OF INTRINSIC ACUTE KIDNEY INJURY

GENERAL PRINCIPLES

Strategies to prevent intrinsic AKI vary based on the specific cause of the kidney injury. Optimization of cardiovascular function and restoration of intravascular volume status are key interventions to minimize the risk that prerenal AKI evolves into ischemic ATN. There is compelling evidence that aggressive intravascular volume expansion reduces the incidence of ATN after major surgery or trauma, burns, and cholera.[53,306,363,364] AKI due to sepsis is common and is associated with mortality rates as high as 80%.[35,296,365] The role of early goal-directed therapy (EGDT) using resuscitation to defined hemodynamic targets (mean arterial pressure [MAP] >65 mm Hg; central venous pressure [CVP], 10–12 mm Hg; urine output >0.5 mL/kg per hour; $ScvO_2$ >70%), using a combination of crystalloid solutions, red cell transfusion, and vasopressors guided by invasive hemodynamic monitoring in improving overall outcomes and decreasing the risk of AKI, has been controversial. In a seminal single-center RCT, EGDT resulted in a significant reduction in overall organ dysfunction and mortality in patients presenting with severe sepsis or septic shock, although specific data on the incidence of AKI were not reported.[366] However, EGDT was not associated with a reduction in dialysis-requiring AKI in the Protocolized Care for Early Septic Shock (ProCESS), Australasian Resuscitation in Sepsis Evaluation (ARISE), or Protocolised Management in Sepsis (PRoMISe) trials or in a patient-level meta-analysis that combined data from these trials (Fig. 29.1).[367–370]

Although the benefits of EGDT were not confirmed in these three later trials, early recognition of sepsis, prompt initiation of antibiotic therapy, and rapid volume resuscitation and hemodynamic stabilization were found to improve outcomes and are likely to minimize the risk of AKI.[371] The role of maintenance of normoglycemia in critically ill patients in minimizing the risk of AKI has also been controversial. Two single-center RCTs that used intensive insulin management to maintain blood glucose levels of 80 to 110 mg/dL, as compared with conventional management maintaining the glucose concentration between 180 and 220 mg/dL, each resulted in decreased rates of AKI, defined either on the basis of change in the serum



No. at risk					
EGDT	1857	1391	1287	1209	1119
Usal care	1880	1395	1295	1206	1110

Fig. 29.1 One-year survival comparing early goal-directed therapy (*EGDT*) with usual care in patients with sepsis. Shown are Kaplan-Meier survival curves comparing EGDT with usual care in patients with sepsis from pooled patient-level data from the ProCESS, ARISE, and ProMISe trials. Renal replacement therapy was required in 11.0% of patients who were randomized to EGDT as compared with 10.6% of patients randomized to usual care (odds ratio, 1.02; 95% confidence interval, 0.81–1.28; P = .88). (From PRISM Investigators; Rowan KM, Angus DC, Bailey, et al. Goal-directed therapy for septic shock—a patient-level metaanalysis. *N Engl J Med.* 2017;376:2223–2234).

creatinine level or on the need for RRT.[372–374] However, the benefits of tight glycemic control were not confirmed in the Normoglycemia in Intensive Care Evaluation–Survival Using Glucose Algorithm Regulation (NICE-SUGAR) trial, a 6104-patient multicenter trial that compared intensive therapy to achieve a target glucose of approximately 80 to 110 mg/dL with more conventional therapy designed to maintain the blood glucose level less than 180 mg/dL.[375] In the NICE-SUGAR trial, intensive glycemic control was associated with an increased risk of hypoglycemia, an increased mortality risk (27.5% vs. 24.9%; P = .02), and no reduction in the need for RRT.

In surgical patients, avoidance of hypotension has been associated with a decreased risk of AKI. In a retrospective analysis of over 33,000 patients who underwent noncardiac surgery, episodes of intraoperative hypotension with a mean arterial blood pressure less than 55 mm Hg were associated with a marked increase in the probability of AKI.[376] The adjusted odds of AKI with intraoperative hypotension increased with the duration of hypotension, from an odds ratio of 1.2 with less than 10 minutes of hypotension to 1.3 with 10 to 20 minutes of hypotension and to 1.5 with more than 20 minutes of hypotension. Volume overload is typical after surgical procedures, and small trials have suggested better patient outcomes following abdominal surgery using a restrictive fluid management strategy. However, a large RCT comparing restrictive and liberal fluid management strategies in patients undergoing major abdominal surgery at increased risk of complications found no benefit with regard to the primary outcome of 1-year disability-free survival, but found a higher rate of AKI associated with the restrictive fluid strategy (8.6% vs. 5.0%; $P < .001$).[377]

Intravascular volume depletion has been identified as a risk factor for ATN resulting from iodinated contrast, rhabdomyolysis, hemolysis, cisplatin, amphotericin B, multiple myeloma, aminoglycosides, and other nephrotoxins, crystal-associated AKI related to acyclovir and acute urate nephropathy, and AKI stemming from hypercalcemia.[74,75,160,169,251,273,364,378–380]

The restoration of adequate intravascular volume prevents the development of experimental and human ATN in many of these clinical settings. Avoidance of potentially nephrotoxic medications or insults in high-risk patients and settings is also important to reduce the risk for ATN. Specifically, among patients with advanced cardiac and/or liver disease in whom renal perfusion may be diminished, the use of selective or nonselective NSAIDs that inhibit the production of vasodilatory prostaglandins may exacerbate intrarenal vasoconstriction and precipitate AKI.[381–385] Diuretics, NSAIDs (including selective cyclooxygenase-2 [COX-2] inhibitors), ACE inhibitors, ARBs, and other inhibitors of the RAAS should be used with caution in patients with suspected absolute or effective intravascular volume depletion or in patients with renovascular disease because these agents may convert reversible prerenal AKI to ischemic ATN. The combined use of agents that block the RAAS, diuretics, and NSAIDs has been identified as a risk factor for AKI, particularly among patients with heart failure, liver failure, or other conditions with reduced baseline renal perfusion.[386,387]

Careful monitoring of circulating drug levels appears to reduce the incidence of AKI associated with aminoglycoside antibiotics and calcineurin inhibitors.[388–390] The observation that the antimicrobial efficacy of aminoglycosides persists in tissues, even after the drug has been cleared from the circulation (postantibiotic killing), has led to the use of once-daily dosing with these agents. Dosing regimens that provide higher peak drug levels but less frequent administration appear to provide comparable antimicrobial activity and less nephrotoxicity than older, conventional dosing regimens.[390–393] Nephrotoxicity of drugs may also be reduced through changes in formulation. For example, the use of lipid-encapsulated formulations of amphotericin B may decrease the risk of amphotericin-induced AKI.[394]

CONTRAST-ASSOCIATED ACUTE KIDNEY INJURY

Contrast-associated AKI (CA-AKI) has historically been defined by and commonly manifests clinically as small absolute (>0.5 mg/dL) and/or relative (>25%) increases in serum creatinine levels that occur within 2 to 4 days following iodinated contrast administration. Although severe AKI is relatively uncommon due to iodinated contrast alone, the incidence of CA-AKI defined by relatively minor increments in the serum creatinine level has been shown in past studies to occur in up to 15% or more of high-risk patients.[395] A series of observational retrospective studies have questioned the true incidence of CA-AKI and challenged the concept that iodinated contrast is nephrotoxic.[396–399] A meta-analysis of 13 nonrandomized studies that included 25,950 patients found a similar risk of AKI in patients who received iodinated contrast IV compared with patients who underwent radiographic procedures without intravascular contrast (relative risk [RR], 0.79; 95% confidence interval [CI], 0.62–1.02).[397] Similarly, an observational study of over 29 million patient hospitalizations found that the risk for AKI among patients who received intravascular iodinated contrast during their hospitalization was comparable to the risk observed among patients who had not received contrast (RR, 0.93; 95% CI, 0.88–0.97).[400] Although the results of these retrospective analyses are adjusted for medical comorbidity and other risk factors for renal injury, such adjustment cannot fully account for all factors that influence the decision to administer contrast, rendering it likely that patients in these studies who did not receive contrast may have been at higher risk for AKI than patients who received contrast. It is therefore prudent to continue to consider intravascular iodinated contrast as potentially nephrotoxic and to implement evidence-based preventive care in patients at risk for CA-AKI (Table 29.8).

Past studies have demonstrated that the administration of IV fluids to high-risk patients prior to and following exposure to intravascular iodinated contrast diminished the risk for CA-AK, although the optimal regimen for fluid administration is unknown.[3,401,402] In a small clinical trial that was stopped early due to safety concerns, periprocedural IV isotonic saline was associated with a markedly lower rate of AKI after contrast exposure than oral fluid administration.[401] In a larger randomized trial, isotonic IV saline significantly reduced the incidence of CA-AKI following coronary angiography compared with half-normal IV saline, with a particular benefit noted in diabetic patients and those receiving large volumes of contrast.[402]

A recent study has challenged the principle that IV fluid administration reduces the risk for CA-AKI.[403] This noninferiority clinical trial randomized 660 patients with baseline CKD undergoing procedures with iodinated contrast to receive isotonic saline IV or no IV fluids and found a similar rate of CA-AKI (2.7% with saline vs. 2.6% without fluid), concluding that withholding IV fluid was not inferior to administering IV saline with regard to the prevention of small increments in the serum creatinine level. However, the patient population in this study was at relatively low overall risk for CA-AKI because more than 50% of participants received IV rather than intraarterial contrast, which is associated with lower rates of CA-AKI, and most had less advanced CKD (i.e., estimated GFR, 45–59 mL/min/1.73 m^2). Furthermore, this trial was powered based on the recruitment of 1300 patients, but enrolled 660 due to feasibility considerations. Thus, until additional clinical trials have demonstrated conclusively that isotonic IV fluid is ineffective, this treatment remains the standard of care for the prevention of CA-AKI.

A number of small clinical trials have compared the effects of isotonic sodium bicarbonate compared with isotonic saline for the prevention of CA-AKI.[404–413] These studies were generally underpowered and yielded conflicting results. Subsequent meta-analyses have concluded that there is an overall benefit associated with bicarbonate administration with regard to AKI defined by small changes in the serum creatinine level, although there was no demonstrable benefit with regard to the need for dialysis.[414–416] This led several clinical practice guidelines to recommend the administration of either IV isotonic sodium chloride or sodium bicarbonate to high-risk patients receiving iodinated contrast.[3,417,418] However, the Prevention of Serious Adverse Events Following Angiography (PRESERVE) trial provided more definitive findings regarding the comparative effects of sodium bicarbonate and sodium chloride for the prevention of CA-AKI.[419] PRESERVE was a multinational randomized clinical trial that used a 2 × 2 factorial design to compare IV isotonic sodium bicarbonate with IV isotonic sodium chloride and oral *N*-acetylcysteine with placebo for the prevention of serious adverse outcomes and CA-AKI following angiographic procedures in 4993 patients with CKD. IV sodium bicarbonate did not reduce the incidence of a primary outcome comprised of 90-day death, need for dialysis, or persistent decline in kidney function (odds ratio [OR], 0.93; 95% CI, 0.72–1.22). Similarly, bicarbonate did not reduce the incidence of CA-AKI assessed 3 to 5 days postangiography (OR, 1.16; 95% CI, 0.96–1.41). Although the optimal rate and duration of IV saline administration is not known, it is reasonable to administer isotonic sodium chloride at a rate of 1 mL/kg per hour for 6 to 12 hours prior to and 6 to 12 hours following a contrast-enhanced procedure in at-risk hospitalized patients. For at-risk outpatients, an alternative regimen of 3 mL/kg over 1 hour prior to the procedure, followed by 6 mL/kg administered over 2 to 6 hours following the procedure, may be more feasible.

Table 29.8 Effectiveness of Preventive Interventions for Contrast-Associated Acute Kidney Injury

No Benefit	Unclear Benefit	Beneficial
Loop diuretics[a]	Statins	Isotonic intravenous fluids
Mannitol[a]	Natriuretic peptides	Low- or iso-osmolal contrast media
Dopamine[a]	Theophylline, aminophylline	
Fenoldopam[a]	Ascorbic acid	
Hemodialysis[a]	Hemofiltration	
N-Acetylcysteine		
Sodium bicarbonate[b]		

[a]Potentially deleterious.
[b]As compared with isotonic saline.

N-acetylcysteine (NAC) is an antioxidant with vasodilatory properties that was postulated to potentially prevent CA-AKI based on its capacity to scavenge reactive oxygen species (ROS), reduce the depletion of glutathione, and stimulate the production of vasodilatory mediators, including nitric oxide.[420,421] Clinical trials of oral and IV NAC have yielded conflicting findings.[422–431] Although initially used at a dose of 600 mg bid,[422] subsequent studies suggested greater efficacy with higher doses of up to 1200 mg bid.[429,430] In the Acetylcysteine for Contrast-Induced Nephropathy (ACT) trial, 2308 patients were randomized to receive 1200 mg of NAC or placebo bid beginning prior to the procedure and continuing for three doses postprocedure.[431] No differences were observed in the incidence of CA-AKI at 48 to 96 hours postcontrast administration or in the incidence of death or need for dialysis within 30 days. However, the overall study population had relatively well-preserved kidney function, with a median serum creatinine level of 1.1 mg/dL, and fewer than 16% of patients had a baseline serum creatinine level more than 1.5 mg/dL. In the aforementioned PRESERVE trial, the administration of NAC in a dose of 1200 mg by mouth bid for 5 days beginning just prior to angiography was not associated with a reduction in 90-day death, need for dialysis, or persistent impairment in kidney function (OR, 1.02; 95% CI, 0.78–1.33) or in a reduction in CA-AKI (OR, 1.06; 95% CI, 0.87–1.28).[419] Based on these findings, NAC should not be used to reduce the risk of CA-AKI.

Trials of other pharmacologic interventions, including furosemide, dopamine, fenoldopam, calcium channel blockers, and mannitol, have failed to demonstrate significant benefit and, in some cases, have been associated with an increased risk of CA-AKI.[432–438] Studies on the benefit of natriuretic peptides, aminophylline, theophylline, and ascorbic acid have also yielded conflicting results.[439–448] Given the absence of convincing data on the efficacy of these interventions, as well as potential safety concerns with the use of natriuretic peptides, aminophylline, and theophylline in patients with cardiovascular disease, their routine use is not recommended.[447] There have been multiple trials and meta-analyses that investigated statins for the prevention of CA-AKI. Although many, albeit not all, have demonstrated a benefit of statins, particularly in a high dose, with respect to the development of CA-AKI, the effect of this class of medication on more serious outcomes, including the need for dialysis and progressive CKD, remains unclear.[449–459] Furthermore, in most patients requiring angiographic procedures, there are likely other indications for statin therapy. RRTs for the prevention of CA-AKI have been largely ineffective and, in some cases, the use of "prophylactic" hemodialysis has been associated with harm.[460–462] The interpretation of studies of hemofiltration for the prevention of CA-AKI is confounded by their use of change in the serum creatinine level as an endpoint, because hemofiltration lowers serum creatinine concentration.[463,464] Given the risks associated with IV line placement and the renal replacement procedures themselves, along with lack of definitive benefit, the use of dialysis or hemofiltration to prevent CA-AKI is not currently recommended[3,465] (Fig. 29.2).

Over the past 25 years, there has been considerable progress in developing less nephrotoxic contrast agents.[466] The use of lower osmolal contrast agents in place of the older and more nephrotoxic high-osmolal agents has resulted in a decreased incidence of CA-AKI.[467,468] Data regarding the added benefit associated with the iso-osmolal radiocontrast agent iodixanol has been less consistent[469–475] and may reflect heterogeneity in the risk of CA-AKI associated with specific lower-osmolal agents.[476]

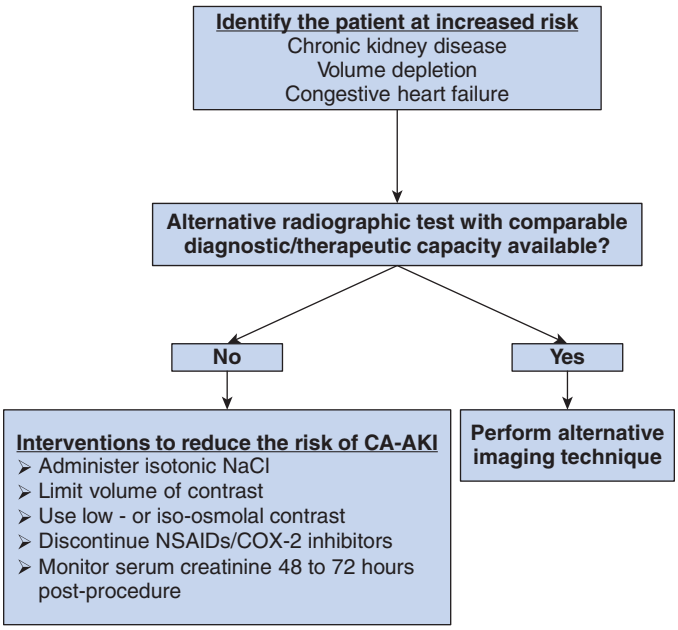


Fig. 29.2 Algorithm for mitigation of risk of contrast-associated acute kidney injury (CA-AKI). *COX-2,* Cyclooxygenase-2; *NSAIDs,* nonsteroidal antiinflammatory drugs.

PREVENTION OF OTHER FORMS OF INTRINSIC ACUTE KIDNEY INJURY

Allopurinol (100 mg/m^2 every 8 hours, maximum, 800 mg/day) is useful for limiting uric acid generation in patients at high risk for acute urate nephropathy. However, AKI can develop despite the use of allopurinol, probably through the toxic actions of hypoxanthine crystals on tubule function.*

In the setting of high rates of uric acid generation, such as tumor lysis syndrome, the use of recombinant urate oxidase (rasburicase, 0.2 mg/kg) may be more effective. Rasburicase catalyzes the degradation of uric acid to allantoin and has been shown to be effective both as prophylaxis and treatment for acute uric acid–mediated tumor lysis syndrome and to prevent the development of AKI due to tumor lysis syndrome–associated hyperuricemia.[164,251,478–481] In oligoanuric patients, prophylactic hemodialysis may be used to acutely lower uric acid levels.

Amifostine, an organic thiophosphate, has been demonstrated to ameliorate cisplatin nephrotoxicity in patients with solid organ or hematologic malignancies.[482–485] NAC limits acetaminophen-induced renal injury if given within 24 hours of ingestion, and dimercaprol, a chelating agent, may prevent heavy metal nephrotoxicity.[486,487] Ethanol inhibits ethylene glycol metabolism to oxalic acid and other toxic metabolites, but its use has been largely replaced by fomepizole, an

*References 160, 251, 271, 273, 477, and 478.

inhibitor of alcohol dehydrogenase that decreases the production of ethylene glycol metabolites and prevents the development of AKI.[488–491]

REMOTE ISCHEMIC PRECONDITIONING

Remote ischemic preconditioning (RIPC) has been investigated as a potential intervention for the prevention of AKI. RIPC involves the implementation of brief episode(s) of ischemia and reperfusion of distant tissue—for example, with sequential brief inflation and deflation of a blood pressure tourniquet on a limb that is hypothesized to enhance the kidneys' resistance to a subsequent more prolonged period of ischemia by means of hormonal mediators, hormonal and neuronal signaling pathways, and antiinflammatory and molecular mediators (Fig. 29.3). Recent trials and meta-analyses have examined the benefits of RIPC in the setting of cardiac surgery. A trial that randomized 240 high-risk patients undergoing cardiac surgery to RIPC or sham RIPC has demonstrated a lower rate of AKI, defined by KDIGO criteria, with RIPC (37.5% vs. 52.5%; $P = .02$), with no effect on secondary endpoints, including myocardial infarction, stroke, and death.[492]

A considerably larger trial that enrolled 1612 patients undergoing cardiac surgery found no difference in the rate of AKI (a secondary endpoint) between RIPC and sham conditioning groups or in the incidence of the combined primary endpoint of death from cardiovascular causes, nonfatal myocardial infarction, coronary revascularization, or stroke within 12 months.[493] A series of meta-analyses of studies that included patients undergoing cardiac, and in some cases, vascular surgery has found lower rates of AKI with RIPC compared with control, but failed to demonstrate a benefit on the need for RRT or death.[494–496] Based on these cumulative data, the use of RIPC for the prevention of adverse outcomes following cardiac and vascular procedures is not routinely recommended.

PHARMACOLOGIC THERAPY FOR ACUTE TUBULAR NECROSIS

During the past 2 decades, there has been extensive investigation into the pathogenesis of AKI using experimental animal models and cultured cells. These studies have resulted in substantial advances in our understanding of the pathophysiology of ATN in humans and led to the discovery of an array of potentially novel targets for the treatment of this common and serious disease. However, multiple interventions shown to ameliorate AKI in animals have failed to be effective in humans with ATN (Box 29.7). There are many possible reasons for the lack of success in translating therapeutic successes for AKI from animal models to clinical practice. Differences in the cause of ATN in animal models and human disease may contribute to differential responses to pharmacologic therapy. Another principal obstacle relates to the difficulty in identifying the incipient stage of ATN prior to elevations in the serum creatinine concentration or clinical evidence of decreased urine output. Over the past decade, several novel serum and urinary biomarkers have been investigated for their ability to identify AKI in its earliest stages and differentiate ATN from volume-responsive AKI.[17] Work in this

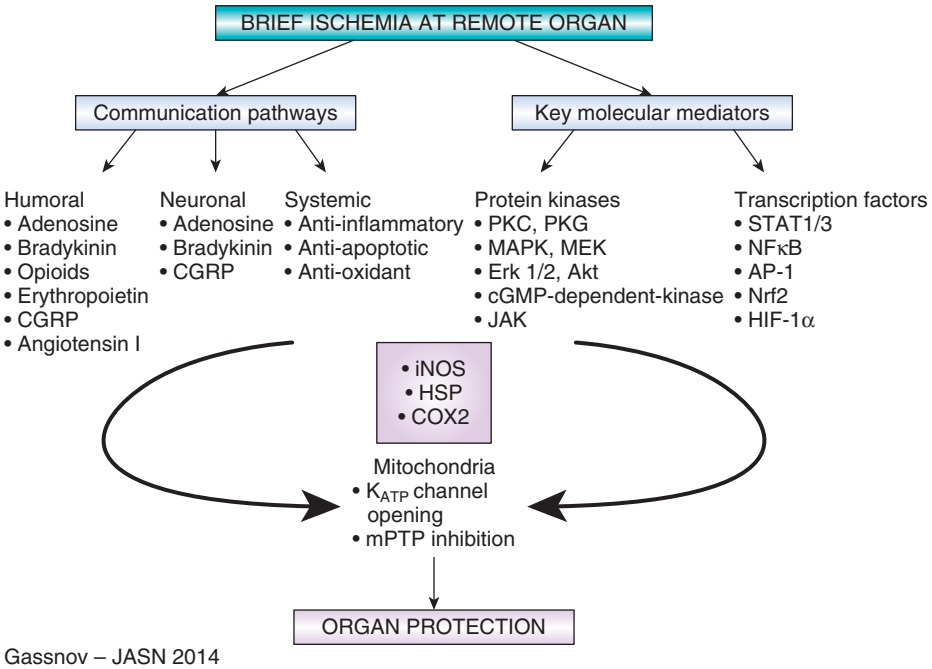


Fig. 29.3 Postulated mechanisms for renal protective effects of remote ischemic preconditioning. *AP-1,* Activator protein-1; *cGMP,* cyclic guanosine monophosphate; *CGRP,* calcitonin gene-related peptide; *COX2,* cyclooxygenase-2; *HIF-1α,* hypoxia-inducible factor 1α; *HSP,* heat shock protein; *iNOS,* inducible nitric oxide synthase; *JAK,* Janus kinase; *MEK,* MAPK kinase; *mPTP,* mitochondrial permeability transition pore; NFkB, nuclear factor kappa B; *Nrf2,* nuclear factor (erythroid-derived 2)-like 2; *PKC,* protein kinase C; *PKG,* protein kinase G; *STAT1/3,* signal transducer and activator of transcription. (From Gassnov N, Nia AM, Caglayan E, Er F. Remote ischemic preconditioning and renoprotection: from myth to a novel therapeutic option? J Am Soc Nephrol. 2014;25(2):216–224.)

Box 29.7 Pharmacologic Interventions Found to Be Ineffective for Acute Tubular Necrosis in Clinical Trials

Diuretics
Dopamine
Fenoldopam
Thyroid hormone
Alpha melanocyte-stimulating hormone
Atrial natriuretic peptide
Alkaline phosphatase
Insulin growth factor
Erythropoietin
Prostaglandin A1

area may facilitate the identification of those patients most likely to respond to treatments that have been found to be effective in animal models.

DOPAMINE

Historically, low-dose ("renal dose") dopamine (<2 mg/kg/min) was widely advocated for the management of oliguric AKI.[497–499] In experimental studies of animals and healthy human volunteers, low-dose dopamine increased renal blood flow and, to a lesser extent, the GFR. However, low-dose dopamine has not been demonstrated to prevent or alter the course of ischemic or nephrotoxic ATN in prospective clinical trials.[500–504] This absence of clinical benefit may relate to differences in the hemodynamic response to low-dose dopamine in patients with renal disease as compared with healthy individuals. In contrast to the reduction in the renal resistive index associated with low-dose dopamine in critically ill patients without kidney disease, dopamine infusion is associated with an increase in renal resistance in patients with AKI.[505] Moreover, dopamine, even at low doses, is potentially toxic in critically ill patients and can induce tachyarrhythmias, myocardial ischemia, and extravasation necrosis.[505] Thus, the routine administration of low-dose dopamine to ameliorate or reverse the course of AKI is not justified based on the balance of experimental and clinical evidence.[506,507]

FENOLDOPAM

Fenoldopam is a selective postsynaptic dopamine agonist that acts on D1 receptors and mediates more potent renal vasodilation and natriuresis than dopamine.[508] However, fenoldopam is a potent antihypertensive agent and causes hypotension by decreasing peripheral vascular resistance. Several small studies have suggested that fenoldopam could reduce the incidence of AKI in high-risk clinical situations[509,510]; however, a subsequent larger randomized trial comparing fenoldopam with standard hydration in patients undergoing invasive angiographic procedures found no benefit in regard to decreasing the incidence of CA-AKI.[435] In another large RCT, fenoldopam administration failed to reduce mortality or the need for renal replacement therapy in ICU patients with early ATN.[511] Therefore, there is currently no clinical role for fenoldopam in the prevention or treatment of AKI.

NATRIURETIC PEPTIDES

ANP is a 28–amino acid polypeptide synthesized in cardiac atrial muscle.[512,513] ANP augments the GFR by triggering afferent arteriolar vasodilation and constriction of the efferent arteriole.[514,515] In addition, ANP inhibits sodium transport and lowers oxygen requirements in several nephron segments.[516,517] Synthetic analogues of ANP have shown promise in the management of ATN in the laboratory setting; however, these benefits in animal models of AKI have failed to translate into clinical benefit in humans. A large multicenter, prospective, randomized, placebo-controlled trial of anaritide, a synthetic analogue of ANP, in patients with ATN failed to show clinically significant improvement in dialysis-free survival or overall mortality,[518] although there was an improvement in dialysis-free survival in oliguric patients. This benefit in oliguric patients was not confirmed in a subsequent prospective study.[519] It has been suggested that the absence of benefit may be related to both the relatively late initiation of therapy and to the effect of ANP on systemic blood pressure. In a subsequent pilot study, low-dose recombinant ANP administration in high-risk cardiac surgery patients was associated with a reduction in the requirement for postoperative RRT.[520] Until these results are confirmed in a larger, multicenter trial, the use of ANP in this setting cannot be recommended. Trials of ANP for the prevention of contrast-associated AKI have generated mixed results.[444,445] Ularitide (urodilantin) is a natriuretic pro-ANP fragment produced in the kidney. In a small randomized trial, ularitide did not reduce the need for dialysis in patients with AKI.[521] A meta-analysis of ANP for the treatment of AKI has concluded that the paucity of high-quality studies precludes a determination of the effects of this therapy.[522]

LOOP DIURETICS

High-dose IV diuretics are commonly prescribed to increase urine output in patients with oliguric AKI. Although this strategy assists in volume management and minimizes the risk of progressive volume overload, there is no evidence that diuretic therapy alters the natural history of AKI or improves mortality or dialysis-free survival. In a retrospective analysis, diuretic therapy was associated with an increased risk of death and nonrecovery of renal function.[523] These risks were restricted, however, to patients who did not respond to diuretic administration with increased urine volume; in diuretic-responsive patients, outcomes were similar to those of untreated patients. In a prospective randomized trial, high-dose IV furosemide augmented urine output but did not alter the outcome of established AKI.[524] In a post hoc analysis of data from the Fluid and Catheter Treatment Trial, a positive fluid balance after AKI in patients with acute lung injury was strongly associated with increased mortality, whereas diuretic therapy was associated with improved 60-day patient survival.[525] Given the risks of loop diuretics in AKI, including irreversible ototoxicity and exacerbation of prerenal AKI, these agents should be used solely to facilitate the management of extracellular volume overload (see later).[526] Of note, a single administration of furosemide in a dose of 1.0 to 1.5 mg/kg has been shown to help characterize the risk for progressive AKI.[527] Specifically, a urine volume less than 200 mL in the 2 hours following furosemide administration demonstrated sensitivity and specificity for progression to AKIN stage 3 of 87.1% and 84.1%, respectively.

MANNITOL

The osmotic diuretic mannitol, which also has renal vasodilatory and oxygen-free radical scavenging properties, has been investigated as a preventive treatment for AKI.[528,529] No adequate data exist to support the routine administration of mannitol to oliguric patients. Moreover, when administered to severely oliguric or anuric patients, mannitol may trigger an expansion of intravascular volume and pulmonary edema, as well as severe hyponatremia due to an osmotic shift of water from the intracellular to the intravascular space.[530]

MANAGEMENT OF OTHER CAUSES OF INTRINSIC ACUTE KIDNEY INJURY

ACUTE VASCULITIS AND ACUTE GLOMERULAR DISEASE

The management of acute vasculitis involving the kidney and acute glomerular disease is covered in detail in Chapters 31 and 32. AKI caused by acute glomerulonephritis or vasculitis may respond to corticosteroids, alkylating agents, rituximab, and plasmapheresis, depending on the primary cause of the disease. Plasma exchange is useful in the treatment of sporadic TTP and possibly sporadic HUS in adults.[531,532] The role of plasmapheresis in drug-induced thrombotic microangiopathies is less certain, and removal of the offending agent is the most important initial therapeutic maneuver.[144,533,534] Postdiarrheal HUS in children is usually managed conservatively because studies have shown that early antibiotic therapy may actually promote the development of HUS.[535] Treatment with eculizumab, a humanized monoclonal antibody that prevents cleavage of complement component C5 into C5a and C5b, inhibiting terminal complement activation, may be considered in patients with nondiarrheal (complement-mediated) HUS unresponsive to plasma exchange.[536] Hypertension and AKI associated with scleroderma may be exquisitely sensitive to treatment with ACE inhibitors.[537–539]

ACUTE KIDNEY INJURY IN MULTIPLE MYELOMA

Early studies have suggested that plasmapheresis may be of benefit in AKI due to myeloma cast nephropathy.[169,540,541] Clearance of circulating light chains, with concomitant chemotherapy to decrease the rate of production, had been postulated to reverse renal injury in patients with circulating light chains, heavy Bence Jones proteinuria, and AKI. A subsequent RCT compared plasma exchange and standard chemotherapy with chemotherapy alone. Although the study did not demonstrate improvement with plasma exchange with regard to a composite outcome of death, dialysis dependence, or a GFR less than 30 mL/min/1.73 m^2 at 6 months, the study was inadequately powered to exclude a clinical benefit definitively, and there was a trend toward improved outcomes with plasmapheresis.[170–172] More recently, it has been suggested that the use of dialysis membranes that are permeable to light chains and other proteins with molecular weights lower than albumin (high-cutoff membranes) may be an effective therapeutic strategy in patients with AKI due to light chain cast nephropathy; however, data from clinical trials have yielded inconclusive results.[173–175] Thus, primary management should focus on the prompt initiation of highly effective chemotherapy. Other contributors to AKI in multiple myeloma, such as hypercalcemia, should also be promptly treated.

ACUTE INTERSTITIAL NEPHRITIS

Most cases of acute interstitial nephritis (AIN) are due to an allergic response to a medication.[542] The initial therapeutic step in AIN is discontinuation of the offending medication or treatment of the probable inciting factor if not drug-induced. Data on the efficacy of corticosteroids have been derived from small observational studies, which have yielded highly discordant results. Although some studies have suggested that early use of corticosteroids (i.e., prior to significant renal damage and within 7–14 days of discontinuation of the offending medication)[543] may be beneficial, other studies have demonstrated no clear evidence of efficacy.[57] There have been no large, prospective RCTs investigating the role of corticosteroids in the treatment of AIN. Because corticosteroids are associated with a series of potentially serious side effects, their use should be considered on a case by case basis. If corticosteroid therapy is being considered and no patient-related contraindications exist, one potential regimen used in one study involved the IV administration of methylprednisolone (250–500 mg/day) for 3 to 4 days followed by oral prednisone at a dose of 1 mg/kg/day tapered over 8 to 12 weeks.[543] However, there are no data supporting the superiority of this specific approach over others. Mycophenolate mofetil has also been investigated as a therapeutic agent for AIN. In a study of eight patients with AIN, six experienced improvement and two experienced stabilization in kidney function with mycophenolate mofetil therapy.[544] Although this small case series suggests a possible role for mycophenolate mofetil in the treatment of AIN, additional studies are needed to confirm its safety and efficacy for this indication.

NONDIALYTIC SUPPORTIVE MANAGEMENT OF ACUTE KIDNEY INJURY–ASSOCIATED COMPLICATIONS

Metabolic complications such as intravascular volume overload, hyperkalemia, hyperphosphatemia, and metabolic acidosis are common in oliguric AKI, and preventive measures should be implemented, beginning with the initial diagnosis (see Table 29.7). Adequate nutrition should be provided to meet caloric requirements and minimize catabolism. In addition, all medications that are normally excreted by the kidney need to be adjusted based on the severity of the renal impairment.

EXTRACELLULAR VOLUME OVERLOAD

After correction of intravascular volume deficits, salt and water intake should be adjusted to match ongoing losses—urinary, gastrointestinal, drainage sites, insensible losses. Extracellular volume overload can usually be managed by restriction of salt and water intake and by judicious use of diuretics. High doses of loop diuretics (e.g., the equivalent of 200 mg of furosemide administered as an IV bolus infusion or 20 mg/hour as a continuous infusion) or combination therapy with both thiazide and loop diuretics may be required. If an adequate diuresis cannot be attained, further use of diuretics should be discontinued to minimize the risk of complications, such as ototoxicity. Fluid administration should be closely monitored to avoid progressive volume overload. Although there is a

strong association between progressive fluid overload and mortality risk in patients with AKI,[545–547] a causal relationship has not been definitively established because volume overload may also be a surrogate for other determinants of mortality, such as hemodynamic instability and capillary leak. Fluid conservative management has, however, been demonstrated to result in improved outcomes in critically ill patients with respiratory failure.[548] Ultrafiltration or dialysis may be required for volume management when conservative measures fail.

HYPONATREMIA AND HYPERNATREMIA

Hyponatremia associated with a fall in effective serum osmolality can usually be corrected by restriction of water intake. Conversely, hypernatremia is treated by the administration of water, hypotonic saline solutions, or hypotonic dextrose-containing solutions (the latter are effectively hypotonic because dextrose is rapidly metabolized).

HYPERKALEMIA

Mild hyperkalemia (<5.5 mmol/L) should be managed initially by restriction of dietary potassium intake and the discontinuation of potassium supplements and potassium-sparing diuretics. More severe degrees of hyperkalemia (5.5–6.5 mmol/L) can usually be controlled by combining these measures with the administration of exchange resins to enhance gastrointestinal potassium losses. Although sodium polystyrene sulfonate has been widely used for decades, concerns have been raised regarding its safety, particularly when administered in 70% sorbitol, due to reports of bowel necrosis.[549,550] Newer exchange resins, patiromer and zirconium cyclosilicate, are also effective at decreasing the serum potassium concentration, although patiromer is not labeled for the acute management of hyperkalemia. Loop diuretics can also increase potassium excretion in diuretic-responsive patients. Emergency measures need to be used in patients with more severe hyperkalemia and in patients with electrocardiographic manifestations of hyperkalemia. In patients with severe hyperkalemia with concomitant electrocardiographic manifestations, the IV administration of calcium will antagonize the cardiac and neuromuscular effects of hyperkalemia and is a valuable emergency temporizing measure, allowing time for the additional measures described later to be implemented. IV calcium must be used with caution, however, if there is concomitant severe hyperphosphatemia or evidence of digitalis toxicity. IV insulin (10–20 U of regular insulin) promotes potassium entry into cells and lowers extracellular potassium concentration within 15 to 30 minutes, with an effect that lasts for several hours.[551,552] The concomitant administration of IV dextrose (25–50 g over 30–60 minutes) is required to prevent hypoglycemia in patients who do not have hyperglycemia. Beta-adrenergic agonists, such as inhaled albuterol (10–20 mg by nebulizer), also promote rapid potassium uptake into the intracellular compartment.[551] Although sodium bicarbonate also stimulates potassium uptake into the intracellular compartment, this effect is not sufficiently rapid to be clinically useful for the emergent management of hyperkalemia.[552] Emergent dialysis is indicated if hyperkalemia is resistant to these measures.

METABOLIC ACIDOSIS

The treatment of metabolic acidosis is dependent on the clinical setting and cause. As a general rule, metabolic acidosis does not require emergent treatment unless the serum HCO_3^- concentration falls below 15 mmol/L or the pH is lower than 7.15 to 7.20. In patients with AKI in whom metabolic acidosis is due to the underlying renal failure, more severe acidosis can be corrected by oral or IV bicarbonate administration. Initial rates of replacement should be based on estimates of the HCO_3^- deficit and adjusted thereafter according to serum levels. In patients with underlying lactic acidosis, the role of bicarbonate therapy is controversial, and the primary focus of therapy should be on correction of the underlying cause.[553–556] Patients treated with IV bicarbonate need to be monitored for complications of therapy, including metabolic alkalosis, hypocalcemia, hypokalemia, hypernatremia, and volume overload.

DISTURBANCES OF CALCIUM, PHOSPHATE, MAGNESIUM, AND URIC ACID

Hypocalcemia does not usually require treatment unless it is severe or symptomatic, as may occur in patients with rhabdomyolysis or pancreatitis or after the administration of bicarbonate. Hyperphosphatemia can often be controlled by restricting dietary phosphate intake and the use of oral phosphate binders (e.g., aluminum hydroxide, calcium salts, sevelamer carbonate, lanthanum carbonate). Caution should be used with aluminum-containing phosphate binders because prolonged use may result in aluminum intoxication, which can contribute to osteomalacia; short-term use is rarely associated with bone disease, and the feared neurologic complications of aluminum intoxication are restricted to patients with inadvertent parenteral exposure. Hypermagnesemia can be prevented through avoidance of magnesium-containing medications, such as antacids, and limiting the magnesium content of parenteral nutrition. Hyperuricemia is usually mild in AKI (<15 mg/dL) and does not require a specific intervention. Severe hyperuricemia secondary to cell lysis may be managed by blocking xanthine oxidase with allopurinol or by enhancing degradation with recombinant uricase, as previously described.

NUTRITIONAL MANAGEMENT

Patients with AKI are clinically heterogeneous, and individualized nutritional management is required, especially in critically ill patients on RRT in whom protein catabolic rates can exceed 1.5 g/kg body weight/day.[3,285,286,288,289,557,558]

The objective of nutritional management in AKI is to provide sufficient calories to preserve lean body mass, avoid starvation ketoacidosis, and promote healing and tissue repair while minimizing production of nitrogenous waste. If the duration of impaired kidney function is likely to be short, the patient is not extremely catabolic and does not require RRT, then dietary protein should be approximately 0.8 to 1.0 g/kg body weight/day.[3] Protein intake should not be restricted in patients in whom AKI is likely to be prolonged, are hypercatabolic, or are receiving RRT. Protein intake in these patients should generally be 1.0 to 1.5 g/kg body weight per day.[285,286,557,558] There has been no evidence of improved outcomes with protein intake higher than 1.7 g/kg body weight per day, even in extremely hypercatabolic patients.[3] Total caloric intake should generally be 20 to 30 kcal/kg body weight per day and should not exceed 35 kcal/kg per day.[3,285,286,557,558] Benefits of vigorous parenteral hyperalimentation have not been consistently demonstrated;

enteral nutrition support is preferred because it avoids the morbidity associated with parenteral nutrition while providing support to intestinal function.[288] Water-soluble vitamins and trace elements should be supplemented in patients receiving RRT.[557,558]

ANEMIA

Severe anemia is generally managed with a blood transfusion. Transfusion is usually not required for patients with a hemoglobin concentration above 7 g/dL.[559] Whether there is a role for erythropoiesis-stimulating agents in AKI has not been definitively determined.[560] Patients with AKI or another acute illness are relatively resistant to the effects of these agents, and their onset of action is delayed. In RCTs in critically ill patients, recombinant human erythropoietin decreased transfusion requirement but had no effect on other outcomes.[561,562] Uremic bleeding usually responds to desmopressin, correction of anemia, estrogens, or dialysis.

DRUG DOSING

Doses of drugs that are excreted by the kidney must be adjusted for impaired kidney function and the use of RRT.[563–565] Whenever possible, pharmacokinetic monitoring should be used to ensure appropriate drug dosing, especially for agents with narrow therapeutic windows (see Chapter 61). In addition to careful monitoring for toxicity of agents that are normally excreted by the kidney, careful attention must be paid to dosing of antibiotics and other drugs removed by RRT to ensure that therapeutic drug levels are achieved, particularly in patients receiving augmented intensity of RRT.

RENAL REPLACEMENT THERAPY IN ACUTE KIDNEY INJURY

GENERAL PRINCIPLES

RRT is the generic term for the multiple modalities of dialysis and hemofiltration used in the management of kidney failure. Although kidney transplantation is also a form of RRT for ESKD, transplantation does not play a role in the management of AKI. Renal replacement therapy facilitates the management of patients with AKI, allowing correction of acid-base and electrolyte disturbances, amelioration of volume overload, and removal of byproducts of nitrogen metabolism (so-called uremic solutes). Although RRT can forestall or reverse the life-threatening complications of uremia associated with severe and prolonged AKI, it does not hasten and can potentially delay the recovery of kidney function in patients with AKI,[566] and can itself be associated with potentially life-threatening complications.[567] Despite more than 60 years of research and clinical experience,[568,569] numerous questions regarding the optimal application of RRT in AKI remain.[3,570–573]

INDICATIONS FOR AND TIMING OF INITIATION OF RENAL REPLACEMENT THERAPY

In clinical practice, there are wide variations in the timing of initiation of RRT for patients with AKI.[574] Widely accepted indications for initiation of RRT include volume overload unresponsive to diuretic therapy, severe metabolic acidosis or hyperkalemia, despite appropriate medical therapy, and overt uremic manifestations, including encephalopathy, pericarditis, and uremic bleeding diathesis (Box 29.8). However, even these specific indications are subject to substantial clinical interpretation. In many patients, RRT is initiated in the absence of these specific indications in response to a clinical course marked by progressive azotemia or sustained oliguria. The correlation between the BUN concentration and the onset of uremic symptoms is relatively weak; although the longer the duration and the greater the severity of azotemia, the more likely it is that overt symptoms will develop. Observational series and small clinical trials dating from the 1950s through the 1980s have suggested that initiating RRT when the BUN concentration approached 90 to 100 mg/dL was associated with improved survival as compared with more delayed initiation of therapy.[575–579] Other observational studies have suggested that the initiation of RRT at an even less severe degree of azotemia may further improve survival.[580–583] These studies need to be interpreted with caution, however, because the outcomes associated with earlier initiation of RRT may reflect differences related to the reasons for initiation of therapy (e.g., volume overload or hyperkalemia vs. progressive azotemia) rather than a benefit due to the earlier therapy per se. In addition, these observational series only included patients in whom RRT was actually initiated, rather than the broader population of patients with AKI, including patients who recovered kidney function or died without receiving RRT.

There have been an increasing number of prospective clinical trials evaluating the timing of initiation of RRT in AKI. In a small RCT of critically ill patients randomized to early, high-volume hemofiltration, early low-volume hemofiltration, or late low-volume hemofiltration, there was no benefit associated with earlier initiation of treatment.[584] In a subsequent trial comparing earlier to later initiation of dialysis in patients with community- acquired AKI, mortality was lower in patients initiated on dialysis later than in the group of patients started earlier, with no difference in recovery of kidney function between groups.[585] This latter trial needs to be interpreted with caution, however, because almost half of the patients admitted with community-acquired AKI were excluded due to the urgent need for dialysis.

More recently, two clinical trials have compared differing strategies of early and delayed initiation of RRT in patients with AKI. The Effect of Early vs. Delayed Initiation of Renal Replacement Therapy on Mortality in Critically Ill Patients with Acute Kidney Injury (ELAIN) trial randomized 231 critically ill patients with AKI (based on KDIGO stage 2 and plasma neutrophil gelatinase-associated lipocalin level >150 ng/mL)

Box 29.8 Indications for Renal Replacement Therapy

Absolute Indications

- Volume overload unresponsive to diuretic therapy
- Persistent hyperkalemia despite medical therapy
- Severe metabolic acidosis
  Overt uremic symptoms—encephalopathy, pericarditis, uremic bleeding diathesis

Relative Indications

- Progressive azotemia without uremic manifestations
  Persistent oliguria

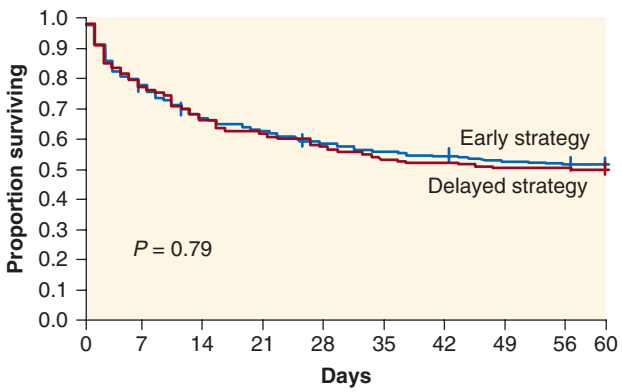
at a single center to early or delayed initiation of RRT; it demonstrated reduced 90-day mortality (hazard ratio, 0.66; 95% CI, 0.45–0.97) with earlier initiation of RRT.[586] Patients in the early RRT group also demonstrated decreased duration of RRT (9 vs. 25 days; $P = .04$) and reduced hospital length of stay (51 vs. 82 days; $P < .001$). However, the separation in timing of the initiation of RRT between early and late groups was less than 1 day, raising questions about the therapeutic mechanisms underlying the observed marked reductions in mortality, duration of RRT, and hospital length of stay. It is possible that unrecognized differences between the two treatment groups may have contributed to the surprisingly large effect size.

The contemporaneous Artificial Kidney Initiation in Kidney Injury (AKIKI) trial was a multicenter trial that randomized 620 patients with KDIGO stage 3 AKI who required mechanical ventilation and/or catecholamine support to early or delayed initiation of RRT. It found no difference in 60-day mortality between the groups (48.5% in the early strategy group vs. 49.7% in the delayed strategy group; $P = .79$)[587] (Fig. 29.4). Notably, nearly half ($n = 151$) of patients assigned to the delayed strategy group never required initiation of RRT. Subsequently, the Initiation of Dialysis Early Versus Delayed in the Intensive Care Unit (IDEAL-ICU) trial also failed to demonstrate a benefit to the earlier initiation of RRT in 488 patients with sepsis-associated AKI.[588] In the IDEAL-ICU trial, patients were enrolled if they met the criteria for RIFLE-F AKI and had sepsis and did not have an emergent indication for RRT. In the early-treatment arm, patients initiated RRT within 12 hours of eligibility while in the delayed-treatment arm; RRT was initiated if a specific indication for RRT developed or if there was no recovery of kidney function after 48 hours. In the early treatment arm, mortality at 90 days was 58% as compared with 54% in the delayed treatment arm ($P = .38$). Of patients in the early treatment arm, 97% received RRT as compared with only 62% of patients in the delayed treatment arm. Of those in the delayed arm who did not receive RRT, 75% had spontaneous recovery of kidney function, whereas 23% died prior to specified criteria for the initiation of RRT.

Key differences between the ELAIN trial and AKIKI and IDEAL-ICU trials should be noted. In particular, the entry criterion for both the AKIKI and IDEAL-ICU trials (stage 3 or RIFLE-F AKI) was the trigger for delayed initiation of RRT in the ELAIN trial. In addition, both the AKIKI and IDEAL-ICU trials excluded individuals with an emergent indication for RRT, in whom a strategy of delayed therapy would be inappropriate. The ongoing Standard vs Accelerated Initiation of RRT in AKI (START-AKI) trial (NCT02568722) may help reconcile the divergent results between the ELAIN trial and AKIKI and IDEAL-ICU trials and clarify the benefit of early initiation of RRT in patients with AKI who do not have overt clinical or biochemical indications for this treatment.[589]

Although volume overload unresponsive to diuretic therapy is a widely accepted indication for the initiation of RRT, there are wide variations in the degree of volume overload at initiation of therapy.[546,590,591] Observational studies have demonstrated a strong association between the degree of volume overload and mortality risk, leading to the suggestion that RRT should be initiated early, prior to the development of progressive volume overload.[545,592] It should be recognized, however, that the association between volume overload and mortality risk does not establish a causal relationship; disease processes that contribute to the development of volume overload may independently contribute to mortality risk in these patients. Prospective studies will therefore be required to demonstrate that preemptive RRT, prior to the development of more severe degrees of volume overload, decreases morbidity and mortality.

Given the current level of evidence, the KDOQI clinical practice guideline for acute kidney injury does not make strong recommendations on the timing of initiation of RRT.[3] It suggests that RRT be "…initiated emergently when life-threatening changes in fluid, electrolyte, and acid-base balance exist",[3] and that "…the broader clinical context, the presence



No. at risk

Early strategy	311	241	207	194	179	172	167	161	158	157
Delayed strategy	308	239	204	191	178	165	161	156	156	155

Fig. 29.4 Probability of survival and timing of treatment with early versus delayed strategies for initiation of renal replacement therapy (RRT). Kaplan-Meier probability of survival and timing of initiation of RRT in the Acute Kidney Initiation in Kidney Injury (AKIKI) trial. In the early-treatment group, 60-day mortality was 48.5% versus 49.7% in the delayed-treatment group (hazard ratio, 1.03; 95% confidence interval, 0.81–1.29; $P = .84$). In the early-treatment group, 98% of patients imitated RRT at a median of 4.3 hours after reaching stage 3 AKI as compared with 51% of patients who initiated RRT at a median of 57 hours. (From Gaudry S, Hajage D, Schortgen F, et al. Initiation strategies for renal-replacement therapy in the intensive care unit. N Engl J Med. 2016;375:122–133.)

of conditions that can be modified by RRT, and trends of laboratory tests—rather than single BUN and creatinine thresholds alone [be considered] when making the decision to start RRT."[3]

RRT should be discontinued when kidney function recovers or because the continued provision of dialytic support is no longer consistent with the patient's overall goals of care.[3] Recovery of kidney function is usually heralded by increased urine volume. Although no specific threshold of urine output correlates with sufficient recovery of kidney function, it is unlikely that a urine output of less than roughly 1 L/day is sufficient to sustain dialysis independence. Although diuretics may increase daily urine volume, there is no evidence that diuretic therapy promotes recovery of kidney function.[593] Improved solute clearance is manifested by a spontaneous fall in blood urea and creatinine concentrations or a persistent downward trend in predialysis values. The role of creatinine clearance measurement to assess the recovery of kidney function is uncertain, with a paucity of data to define specific thresholds for recovery of kidney function. In the Acute Renal Failure Trial Network Study, RRT was continued if measured creatinine clearance on a 6-hour timed urine collection was less than 12 mL/min, RRT was stopped if the clearance was more than 20 mL/min, and the decision was left to the discretion of the clinician if the creatinine clearance was between 12 and 20 mL/min.[594,595]

CHOICE OF MODALITY OF RENAL REPLACEMENT THERAPY

Multiple modalities of RRT are available for the management of patients with AKI, including conventional intermittent hemodialysis (IHD), peritoneal dialysis (PD), multiple forms of continuous renal replacement therapy (CRRT) and prolonged intermittent renal replacement therapies (PIRRT) such as sustained low-efficiency dialysis (SLED; also known as extended- duration dialysis, EDD). Detailed descriptions of the technical aspects of these modalities are provided in Chapters 63, 64, and 65. Objective data to guide the selection of modality for individual patients are limited, and the choice of modality is often guided by the resources of the health care institution and the technical expertise of the physicians and nursing staff. The KDIGO Clinical Practice Guideline for Acute Kidney Injury suggests that for most patients, the available modalities of RRT are complementary, with the caveats that CRRT and PIRRT be used in hemodynamically unstable patients and that CRRT be used for patients with acute brain injury or other causes of increased intracranial pressure or generalized brain edema.[3]

Intermittent Hemodialysis

IHD has been the mainstay of RRT in AKI for more than 6 decades. Patients typically undergo dialysis treatments for 3 to 5 hours on a thrice-weekly, alternate-day, or daily schedule, depending on catabolic demands, electrolyte disturbances, and volume status. Just as with the timing of initiation of dialysis in AKI, the most appropriate dosing strategy for IHD in patients with AKI has been the subject of considerable investigation. The dose of IHD may be adjusted by altering the intensity of each individual dialysis session, usually quantified as the product of urea clearance and dialysis duration normalized to the volume of distribution of urea (Kt/V_{urea}) or by changing the frequency of the dialysis sessions. In an observational study, Paganini and colleagues have demonstrated a survival benefit for patients with intermediate severity of illness scores when the delivered Kt/V_{urea} was more than 1.0 per treatment as compared with a delivered Kt/V_{urea} less than 1.0 per treatment.[596]

However, there have been no prospective clinical trials evaluating the relationship between the delivered Kt/V_{urea} and outcomes when dialysis is provided on a constant treatment schedule. Schiffl and colleagues have reported on a prospective trial of 160 patients with AKI assigned in an alternating fashion to alternate-day or daily intermittent hemodialysis.[597] The more frequent treatment schedule was associated with a reduction in mortality at 14 days after the last dialysis session, from 46% in the alternate-day dialysis arm to 28% in the daily treatment arm ($P = .01$). The duration of renal failure declined from 16 ± 6 to 9 ± 2 days ($P = .001$). This study has been criticized, however, because the delivered dose of therapy per session was low in both treatment arms ($[Kt/V_{urea}] < 0.95$), resulting in a high rate of symptoms in the alternate-day dialysis arm that may have been related to overtly inadequate dialysis.[598]

The impact of frequency of IHD was also evaluated in the Acute Renal Failure Trial Network study.[594] In this study, 1124 critically ill patients were randomized to an intensive or less intensive strategy for the management of RRT. When patients were hemodynamically stable, they received IHD and, when hemodynamically unstable, they received CRRT or sustained low-efficiency dialysis (SLED), regardless of treatment arm. Patients randomized to the less intensive treatment strategy received IHD on a thrice-weekly (alternate-day except Sunday) schedule while patients randomized to the intensive arm received IHD six times per week (daily except Sunday). All-cause mortality at 60 days was 53.6% in the intensive treatment arm compared with 51.5% in the less intensive arm ($P = .47$)[594] (Fig. 29.5). The mean delivered Kt/V_{urea} was 1.3/treatment after the first IHD session. Although the study was not designed to evaluate outcomes by individual modality of RRT, there were no differences in mortality between groups when evaluated based on the percentage of time treated using IHD.[599]

Based on these results, it does not appear that there is further benefit to increasing the frequency of IHD treatments routinely beyond three times per week as long as the delivered Kt/V_{urea} is at least 1.2 per treatment. More frequent treatments may be necessary if the target dose per treatment cannot be achieved—for example, in hypercatabolic patients, in patients with severe hyperkalemia or metabolic acidosis, and for issues related to volume management. The KDIGO Clinical Practice Guideline for Acute Kidney Injury recommends delivering a Kt/V_{urea} of 3.9 per week when using IHD in AKI, calculating the weekly Kt/V_{urea} as the arithmetic sum of the delivered dose per treatment.[3] It should be recognized, however, that this approach for calculating an equivalent weekly Kt/V_{urea} is not consistent with urea kinetic principles, and that rigorous data for the appropriate dose of therapy when treatments are delivered more frequently than three times per week are not available.[13]

The selection of IHD dialyzer membrane may also affect clinical outcomes. Exposure to cellulosic membranes results in accentuated leukocyte and complement activation and delayed recovery of kidney function in experimental models of AKI as compared with exposure to more biocompatible

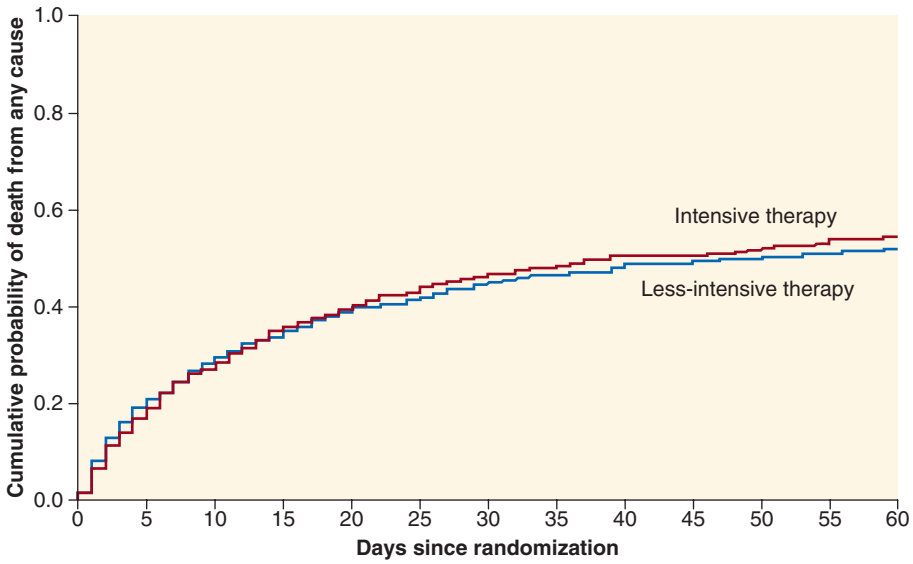


Fig. 29.5 60-Day mortality with intensive versus less intensive renal replacement therapy in the Acute Renal Failure Trial Network (ATN) study. Shown is the Kaplan-Meier plot of mortality in 1124 critically ill patients with acute kidney injury randomized to a strategy of more intensive renal replacement therapy (RRT; 6 times per week intermittent hemodialysis or continuous venovenous hemodiafiltration at 35 mL/kg per hour) versus less intensive RRT (3 times per week intermittent hemodialysis or continuous venovenous hemodiafiltration at 20 mL/kg per hour). At 60 days, mortality was 53.6% in the more intensive arm versus 51.5% in the less intensive arm (odds ratio, 1.09; 95% confidence interval, 0.86–1.40; $P = .47$). (From VA/NIH Acute Renal Failure Trial Network; Palevsky PM, Zhang JH, O'Connor TZ, et al. Intensity of renal support in critically ill patients with acute kidney injury. N Engl J Med. 2008;359:7–20.)

synthetic membranes.[600,601] Clinical trials comparing dialysis membranes have yielded conflicting results. Although some studies have demonstrated delayed recovery of kidney function with cellulosic membranes,[602–604] other studies have observed no difference between cellulosic and other synthetic membranes thought to be more biocompatible.[605–609] When these data have been aggregated in systematic reviews, a benefit of the synthetic membranes is not convincingly demonstrated.[610,611] Although the effect of membrane type on humoral and cellular activation may still influence recovery of kidney function in AKI, the clinical importance of this issue has diminished as the cost differential between synthetic and cellulosic membranes has narrowed, and the use of unsubstituted cellulosic membranes has decreased.

The major complications associated with IHD are related to the need to access the vasculature, the need for anticoagulation to maintain patency of the extracorporeal circuit, and intradialytic hypotension primarily resulting from shifts in solute and volume.[594,597,612] Many of these issues, particularly the need for vascular access and anticoagulation, are similar for CRRT and SLED.

Vascular access is usually obtained through the insertion of a double-lumen catheter into a large caliber central (internal jugular or subclavian) or femoral vein.[613] The major complications associated with vascular access include vascular and organ trauma during insertion, bleeding, catheter malfunction and thrombosis, and infection.[613] Although femoral catheters are generally associated with an increased risk of infection compared with catheters in the subclavian or internal jugular veins, an increased risk of infection was observed only when femoral vein catheters were used in patients with a high body mass index (BMI) in an RCT involving patients undergoing acute RRT.[614] The more prompt transition to tunneled hemodialysis catheters (or placement of tunneled hemodialysis catheters in advance of initiation) has been proposed as a means of decreasing the risk of infection in patients undergoing acute dialysis.[615,616] However, this strategy has not been rigorously evaluated in prospective clinical trials.

Anticoagulation is used to help maintain patency of the extracorporeal dialysis circuit in IHD, as well as in CRRT and SLED.[617,618] The most commonly used anticoagulant for dialysis is unfractionated heparin; with multiple protocols used to attain sufficient anticoagulation of the dialysis circuit while minimizing systemic effects.[617,618] Regional heparinization, in which heparin is infused proximally to the dialyzer, and protamine is infused into the return line to reverse heparin's effect,[619] can be used but has generally been supplanted by low-dose heparin protocols.[620] Low-molecular-weight heparin (LMWH) may be used as an alternative to unfractionated heparin; however, the benefits of this approach are unclear because LMWH is not associated with enhanced efficacy, drug half-life is variably prolonged with impaired kidney function, and monitoring of the anticoagulant effect is more difficult.[617] In patients with heparin-induced thrombocytopenia (HIT), heparin administration is contraindicated. Alternative anticoagulation strategies include regional citrate,[617,621–623] the serine protease inhibitor nafamostat,[624] the direct thrombin inhibitors hirudin, lepirudin, and argatroban[625–629] and, rarely, the prostanoids epoprostenol and iloprost.[617,618] In many patients, particularly those with underlying coagulopathy or thrombocytopenia, and in patients with active hemorrhage or recent postoperative status, acute RRT can be provided in the absence of anticoagulation.[594,630,631]

Intradialytic hypotension is common in patients undergoing acute IHD.[566,594,599,612,632] Episodes of hypotension may impair solute clearance and the efficiency of dialysis and can further

compromise renal perfusion and delay recovery of kidney function.[566,633–635] Intradialytic hypotension is typically triggered by intercompartmental fluid shifts or excessive fluid removal, leading to decreased intravascular volume, and may be exacerbated by altered vascular responsiveness related to the underlying acute process.[612,636] Hypotension may be particularly problematic in critically ill patients in whom sepsis, cardiac dysfunction, hypoalbuminemia, malnutrition, or large third space losses may accompany the development of AKI. The prevention of intradialytic hypotension requires careful assessment of intravascular volume, prescription of realistic ultrafiltration targets, extension of treatment time so as to minimize the ultrafiltration rate, increasing the dialysate sodium concentration, and decreasing the dialysate temperature.[632,636–638] It is noteworthy that intradialytic hypotension can develop even among patients in whom no ultrafiltration is prescribed; the reason(s) for hypotension are not entirely clear, although many have attributed hemodynamic instability to the rapid exchange of solutes induced by high-flux, high-efficiency hemodialysis, with extra- to intracellular shifting of body water. Although there is a tendency to reduce the extracorporeal blood flow in patients prone to hypotension, there is little evidence that this provides any benefit. Reducing blood flow decreased the volume of the extracorporeal circuit in the past when parallel plate and coil dialyzers were used; however, there is little change in the volume of the extracorporeal circuit in response to changes in blood flow when hollow fiber dialyzers are used. Reducing blood flow may, however, result in reduction of the delivered dose of dialysis.

Continuous Renal Replacement Therapy

The continuous RRTs represent a spectrum of treatment modalities. In their initial description, the continuous therapies were provided using arteriovenous extracorporeal circuits.[639–643] Although this approach provided technical simplicity, blood flow was dependent on the gradient between MAP and central venous pressure, and there was an increased risk of complications from prolonged arterial cannulation.[644] As a result, the continuous arteriovenous therapies have largely been supplanted by pump-driven, venovenous CRRT.[645–648] The modalities of venovenous CRRT vary, predominantly based on their mechanism of solute removal. With continuous venovenous hemofiltration (CVVH), solute transport occurs by convection; with continuous venovenous hemodialysis (CVVHD) by diffusion; and with continuous venovenous hemodiafiltration (CVVHDF) by a combination of the two.[648–650] Although, at the same level of urea clearance, convective therapies provide enhanced clearance of higher molecular weight solutes compared with diffusive therapies, no clear clinical benefit has been demonstrated for CVVH or CVVHDF as compared with CVVHD.[651]

The clearance of urea and other small solutes during CRRT is generally proportional to the total effluent flow rate (the sum of ultrafiltrate and dialysate flow rates),[643,648,649] and the dose of therapy is usually expressed as the effluent volume indexed to body weight. This approach to estimating solute clearance is based on the assumption of near-complete solute equilibration between blood and effluent and may overestimate the actual solute clearance.[652,653] Several single-center RCTs demonstrated an improvement in survival when doses of CVVH were increased from 20 to 25 mL/kg per hour to doses in excess of 35 to 45 mL/kg per hour[654,655]; however, other small studies did not find a similar benefit.[584,656] Two large multicenter RCTs also did not find a survival benefit associated with more intensive CRRT.[594,657] In the previously described ATN study, 1124 patients were randomized to two intensities of RRT.[594] In both treatment arms, patients received IHD when hemodynamically stable and CVVHDF or SLED when hemodynamically unstable. In the less intensive arm, CVVHDF was provided at an effluent flow rate of 20 mL/kg per hour and,in the more intensive arm at 35 mL/kg per hour. All-cause mortality at 60 days was 51.5% in the less intensive arm and 53.6% in the more-intensive arm (P = .47; Fig. 29.5).[594] In the Randomized Evaluation of Normal versus Augmented Level (RENAL) Replacement Therapy study, 1508 patients were randomized to CVVHDF at 25 mL/kg or 40 mL/kg per hour.[657] All-cause mortality at 90 days was 44.7% in both treatment arms (P = .99; Fig. 29.6).[657] Based on these data, the KDIGO Clinical Practice Guideline for Acute Kidney Injury recommends delivering an effluent volume during CRRT of 20 to 25 mL/kg per hour, recognizing that a slightly higher dose may need to be prescribed to achieve the target delivered dose to compensate for interruptions in treatment.[3]

Given the improved hemodynamic tolerance of CRRT as compared with IHD, particularly in patients with underlying hemodynamic instability, it has been postulated that CRRT would be associated with improved clinical outcomes. Five RCTs comparing outcomes with CRRT and IHD have been published. In a multicenter RCT of 166 patients with AKI, Mehta and colleagues observed ICU and hospital mortality rates of 59.5% and 65.5%, respectively, in patients randomized

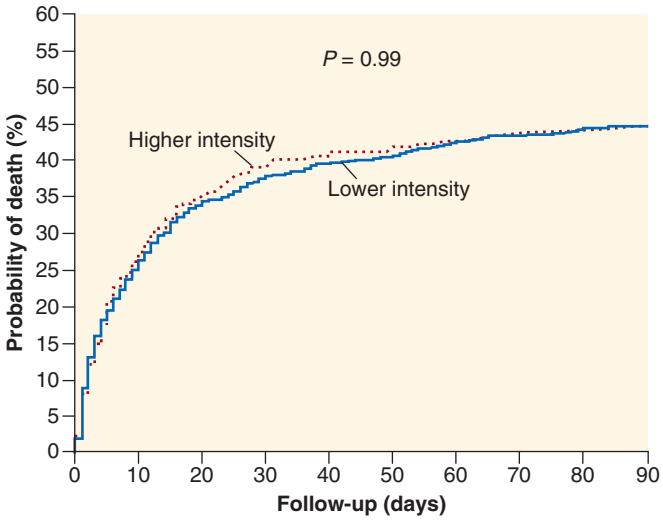
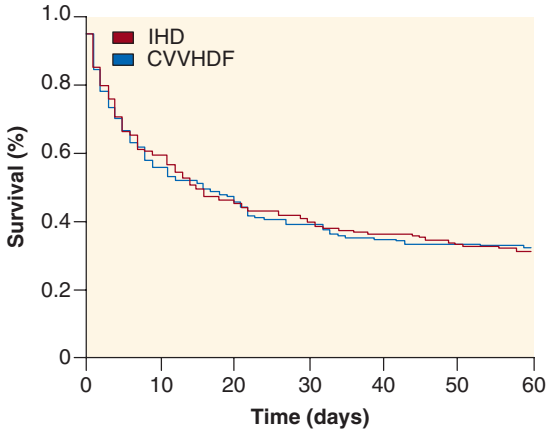


Fig. 29.6 90-Day mortality with intensive versus less intensive continuous venovenous hemodiafiltration (CVVHDF) in the Randomized Evaluation of Normal versus Augmented Level (RENAL) Replacement Therapy Study. Shown is the Kaplan-Meier plot of mortality in 1508 critically ill patients with acute kidney injury randomized to CVVHDF at 35 mL/kg vs. 20 mL/kg per hour. At 90 days, mortality was 44.7% in both treatment groups (odds ratio, 1.00; 95% confidence interval, 0.81–1.23; P = .99). (From RENAL Replacement Therapy Study Investigators; Bellomo R, Cass A, Cole L, et al. Intensity of continuous renal-replacement therapy in critically ill patients. N Engl J Med. 2009;361:1627–1638.)

to CRRT as compared with 41.5% and 47.6%, respectively, in patients randomized to IHD ($P < .02$).[658] As the result of an imbalance in randomization, patients in the CRRT arm had a higher severity of illness as measured by the APACHE III score and a higher rate of liver failure. Adjusting for the imbalanced randomization in a post hoc analysis, the investigators found no difference in mortality attributable to modality of RRT. In another single-center randomized trial ($n = 80$), Augustine and colleagues reported more effective fluid removal and greater hemodynamic stability associated with CVVHD as compared with IHD, but no difference in survival.[659] Similarly, in another single-center RCT from Switzerland, Uehlinger and colleagues observed no difference in survival in 70 patients randomized to CVVHDF as compared with 55 patients assigned to IHD.[660] In the Hemodiafe study, a multicenter RCT conducted in 21 ICUs in France, Vinsonneau and colleagues reported 60-day survival rates of 31.5% in 184 patients randomized to IHD as compared with 32.6% in 175 patients randomized to CVVHDF ($P = .98$)[632] (Fig. 29.7). Similarly, Lins and colleagues observed hospital morality rates of 62.5% in 144 patients randomized to IHD and 58.1% in 172 patients randomized to CRRT ($P = .43$).[661] Multiple meta-analyses have concluded that there is no difference in survival among patients undergoing RRT.[662–664] Although several studies have suggested that CRRT is associated with improved rates of recovery of kidney function in surviving patients as compared with IHD,[658,665–668] all these studies are confounded by higher mortality rates in the CRRT group. When analyzed across studies in which there were no differences in mortality, rates of recovery of kidney function did not appear to be affected by the modality of RRT.[566,662,664,669]



Number at risk

IHD	184	85	68	58
CVVHDF	175	83	62	57

Fig. 29.7 60-Day survival with intermittent hemodialysis (*IHD*) versus continuous venovenous hemodiafiltration (*CVVHDF*) in the Hemodiafe Study. Shown is a Kaplan-Meier plot of survival among 359 critically ill patients with acute kidney injury randomized to intermittent hemodialysis versus continuous venovenous hemodiafiltration. At 60 days,survival was 31.5% among patients randomized to IHD versus 32.6% among patients randomized to CVVHDF ($P = .98$). (From Vinsonneau C, Camus C, Combes A, et al; Hemodiafe Study Group. Continuous venovenous haemodiafiltration versus intermittent haemodialysis for acute renal failure in patients with multiple-organ dysfunction syndrome: a multicentre randomised trial. Lancet. 2006; 368:379–385.)

Prolonged Intermittent Renal Replacement Therapy

Prolonged intermittent renal replacement therapy (PIRRT) represents a treatment modality in which conventional hemodialysis equipment is modified to provide extended-duration hemodialysis using lower blood flow rates and dialysate flow rates.[670,671] A variety of terms have been developed to describe these therapies including SLED,[672,673] extended daily dialysis (EDD),[674] and sustained low-efficiency daily diafiltration (SLEDD-f).[675] By extending the duration of the dialysis treatment while providing slower ultrafiltration and solute clearance, these therapies are generally associated with enhanced hemodynamic tolerability compared with IHD. The degree of metabolic control attained with these treatments is comparable to that observed with CRRT.[676] In an observational study performed in three ICUs in New Zealand, Australia, and Italy that changed from using CRRT to PIRRT, there was no difference in observed outcomes following the change in modality of RRT.[671] Similarly, in a single-center prospective RCT that included 232 patients, 90-day survival rates were similar in the PIRRT and CRRT groups (PIRRT, 50.4%; CRRT, 44.4%; $P = .43$), although overall resource use was lower with PIRRT.[677] In a meta-analysis, there were no differences in mortality or recovery of kidney function comparing PIRRT with CRRT.[678]

Peritoneal Dialysis

The use of PD in the management of AKI has diminished as the use of continuous and hybrid therapies have increased.[679–681] Peritoneal dialysis has the advantage of requiring minimal technology, facilitating its use in remote or resource-constrained areas.[682] As a result, it is still used in the treatment of AKI in regions where access to IHD or CRRT is not possible. Access for acute PD can be obtained by percutaneous placement of an uncuffed temporary peritoneal catheter or through surgical placement of a tunneled cuffed catheter. PD has the advantage of avoiding the need for vascular access or anticoagulation. Solute clearance and control of metabolic parameters may be inferior to that achieved with other modalities of RRT.[683] Although systemic hypotension is less of an issue than with other modalities of RRT, ultrafiltration cannot be as tightly controlled. Other limitations include the relative contraindication in patients with acute abdominal processes or recent abdominal surgery, the risk of visceral organ injury during catheter placement, the risk of PD-associated peritonitis, and an increased tendency toward hyperglycemia due to the high glucose concentrations in peritoneal dialysate, which in other acute settings has been associated with adverse outcomes.

Several trials have compared outcomes using PD with other modalities of RRT in AKI.[683–686] In a study of 70 patients with infection-associated AKI in Vietnam, 58 of whom had severe falciparum malaria, PD was associated with less adequate metabolic control and higher mortality than continuous hemofiltration.[683] In contrast, in a study of 120 patients in Brazil who were randomized to high-volume PD or daily hemodialysis, indices of metabolic control, recovery of kidney function, and survival were similar with both modalities.[684] In a meta-analysis that included eight observational studies and four clinical trials, Chionh and colleagues observed similar survival rates with peritoneal dialysis as compared with extracorporeal RRT in patients with AKI.[687]

SUMMARY OF ACUTE KIDNEY INJURY MANAGEMENT

Acute kidney injury remains a common and serious disease with protean causes and variable clinical courses. The management of AKI begins with prevention in those clinical circumstances in which evidence-based preventive interventions are available. Although pharmacologic therapy is available for select causes of AKI, the treatment of established ATN is largely supportive, with pharmacology and RRTs designed to ameliorate the adverse metabolic and clinical complications of this condition. Additional research is needed to identify treatments that decrease the risk of developing AKI and, in patients with established AKI, reduce the severity of AKI and/or facilitate recovery.

Complete reference list available at ExpertConsult.com.

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BOARD REVIEW QUESTIONS

1. Which of the following is true regarding the role of early goal-directed therapy (EGDT) for the management of sepsis?
   a. EGDT is associated with a reduction in dialysis-requiring acute kidney injury (AKI) but not mortality.
   b. EGDT is associated with a reduction in dialysis-requiring AKI and mortality.
   c. Recent clinical trials have demonstrated that EGDT is not associated with a reduction in dialysis-requiring AKI.
   d. Early antibiotic therapy and volume resuscitation are not associated with improved patient outcomes.

   Answer: c

   Rationale: The recent ProCESS, ARISE, and ProMISE trials did not demonstrate a reduction in the need for renal support with EGDT.

2. A 63-year-old woman with diabetes mellitus complicated by retinopathy and nephropathy is admitted with a non–ST-elevation myocardial infarction. Her serum creatinine level is 1.68 mg/dL and her urine albumin excretion is 762 µg/mg creatinine. Which one of the following interventions is associated with the strongest evidence for reducing her risk of developing contrast-associated acute kidney injury following her planned coronary angiography?
   a. Oral *N*-acetylcysteine
   b. Periprocedural hemofiltration
   c. Periprocedural infusion of isotonic sodium chloride
   d. Use of iodixanol

   Answer: c

   Rationale: Periprocedural infusion of isotonic sodium chloride is the strongest evidence based on the results of the PRESERVE trial, absence of sound evidence of any benefit with hemofiltration, and studies demonstrating no difference between iodixanol and low osmolal contrast agents with regard to risk for AKI.

3. Which one of the following strategies for the management of renal replacement therapy in patients with acute kidney injury and multisystem organ failure in the setting of sepsis is most strongly supported by evidence from clinical trials?
   a. Use of continuous renal replacement therapy rather than intermittent hemodialysis
   b. Use of continuous hemofiltration rather than continuous hemodialysis
   c. Use of lower doses (20–25 mL/kg per hour) rather than higher-doses (>35 mL/kg per hour) of continuous renal replacement therapy
   d. Use of daily intermittent hemodialysis with a delivered Kt/V_{urea} of 1.2 to 1.4 per treatment rather than alternate-day hemodialysis with the same delivered Kt/V_{urea} per treatment

   Answer: c

   Rationale: Use of lower doses (20–25 mL/kg per hour) rather than higher-doses (>35 mL/kg per hour) of continuous renal replacement therapy is the correct option based on the results of the ATN and RENAL trials and consistent with the KDIGO AKI Clinical Practice Guidelines.

4. With regard to the optimal time to initiate renal replacement therapy in the setting of acute kidney injury, which of the following is true?
   a. No clinical trials have compared early versus late initiation of renal replacement therapy for acute kidney injury.
   b. Early initiation of renal replacement therapy is associated with improved recovery of kidney function and lower mortality.
   c. The costs of early initiation of renal replacement therapy outweigh the potential clinical benefit.
   d. There are currently insufficient data to support the clinical benefit of early initiation of renal replacement therapy.

   Answer: d

   Rationale: Trials to date have generated conflicting data on the benefit of early initiation of renal replacement therapy.

30 Pathophysiology of Proteinuria

Norberto Perico | Andrea Remuzzi | Giuseppe Remuzzi

CHAPTER OUTLINE

MECHANISMS OF PROTEINURIA

One of the most common features of glomerular diseases is an abnormal excretion of plasma proteins in the urine. Proteinuria is the cause and effect of several complications not only at a kidney but also at a systemic level. There are complex changes in the structure and function of the glomerular capillary, as well as in the entire nephron, that are responsible for the final elevation in urine protein concentration in several kidney disorders. In this chapter, before describing the consequences of proteinuria, the pathophysiology of protein excretion is reviewed.

The functional properties of the glomerular filtration barrier, tubular interaction with filtered proteins and the mechanisms of proteinuria have been reviewed in detail,[1] and the characterization of structural molecules relevant to the filtration barrier in glomerular endothelial cells and in particular on podocytes has been reported.[2,3] There are, in principle, two distinct phenomena that can result in proteinuria. The first is the elevation of glomerular filtration of circulating plasma proteins that are almost completely retained in the circulating plasma in normal physiologic conditions; the second is a defective or incomplete reabsorption of proteins by the proximal tubule. The two phenomena are interrelated, and likely are both present in so-called glomerular proteinuria, when proteins the size of albumin and larger are present in urine. Despite several experimental and clinical observations investigating the structural and molecular alterations involved in kidney diseases resulting in proteinuria,[4] the precise nature of the functional changes responsible and their quantification remain the subject of numerous ongoing investigations.[5]

STRUCTURE AND FUNCTION OF THE GLOMERULAR CAPILLARY WALL

The function of the glomerular capillary is to allow a large amount of water and small solute filtration while efficiently restricting glomerular passage of protein macromolecules within blood circulation. This selective function is specific to the glomerular capillary membrane, which is far more permeable to water than any other capillary membrane in the body. With the development of glomerular diseases, the capillary membrane structure at molecular and/or cellular level may be altered, resulting in loss of hydraulic permeability, reduction in surface area available for filtration, and consequent loss of glomerular filtration rate (GFR). Despite the reduction in permeability to water, the capillary membrane often becomes more permeable to circulating macromolecules. Experimental research has elucidated a number of glomerular structural and molecular alterations that are responsible for these functional changes.

GLOMERULAR CAPILLARY WALL ORGANIZATION

Morphologic studies available in the literature describe in detail the complex organization of the glomerular capillary and the capillary membrane (see Chapter 2). However, the interpretation of filtration barrier function has been largely based on major simplifications. Thus although the glomerular capillary is composed of numerous branching segments, the glomerular capillary organization has usually been considered as a simple capillary segment or as a set of several uniform segments in parallel. Similarly, the capillary membrane has been considered as a uniform three-layer structure. As described later, recent investigations allow a better understanding of the functional effects of the geometric and spatial organization of the glomerular capillary, as well as specific features of cell organization and interactions at the capillary membrane level. These aspects have revealed some new insights in the mechanisms responsible for glomerular capillary dysfunction.

Glomerular Capillary Network

According to classic optical microscopy observations of kidney tissue sections, the capillary network is composed of a number of capillary segments connecting afferent and efferent arterioles within a tuft that, in humans, has a mean diameter of 120–150 μm. More realistic and direct visualizations of the

capillary organization are usually derived from scanning electron microscopy, but this technique allows predominantly only views from the outer surface of the capillary. Specific investigations with reconstructions from serial sections[6] or confocal microscopy[7] allow investigation of the capillary segment organization and, in particular, calculation of blood flow distribution and water filtration along the network.[8] Due to the large number of capillary segments, around 200 in the rat, and their apparent uniform size, the blood flow is expected to be uniformly distributed along the network with lower blood velocity as compared with that in the afferent arteriole. This hemodynamic arrangement allows the blood to remain in close contact with the filtration membrane. However, more detailed geometric reconstructions of the glomerular capillary show that the network has some heterogeneity. The size of some capillary segments (with diameters less than 3–4 μm)[6] would suggest that they are perfused only by plasma, excluding red blood cell transit, and may represent a sort of shunt in the network to decrease overall network pressure. This finely organized geometry is the result of cellular organization and remodeling and seems to be importantly affected by disease processes, resulting in simplification of capillary network, changes in local pressure and flow distribution, ultimately resulting in capillary obliteration in areas of segmental sclerosis.[9] These local hemodynamic changes affect the filtration function of the capillary network, as elevation of blood flow and hydraulic pressure is expected to occur in some capillary segments leading to abnormal filtration of circulating proteins.[10]

Glomerular Capillary Wall

At a smaller scale the organization of the glomerular capillary membrane is rather heterogeneous. The arrangement that is generally described usually refers to the portion of the capillary wall that is considered the filtering surface, characterized by a three-layer composition consisting of endothelial cells, glomerular basement membrane (GBM), and epithelial cells. The structure and function of this highly differentiated arrangement of cells and matrix is presented in Chapter 2. Mechanisms whereby structural and functional changes may result in abnormal protein filtration and ultimately in proteinuria are reviewed later.

According to the classic concept, hydraulic resistance and macromolecule retention are functions of the so-called filtering surface of the glomerular capillary membrane, but recent evidence indicates that the entire structure of the epithelial cells and the relative position of the capillary membrane within the tuft may also affect water and macromolecule filtration. As reported by Neal et al.,[11] a large fraction of the filtration membrane is covered by epithelial cell bodies or by the presence of adjacent epithelial cells. The three-dimensional spaces created by these structures have been called subpodocyte (SPS) and interpodocyte spaces (IPS), respectively. Theoretical analysis of the transport of both water and macromolecules through the SPS[12] indicates that the structural organization of this compartment induces significant resistance to water flow from the filtration membrane to the urinary space. This resistance appears to not be insignificant as compared with that of the three-layer membrane structure. Macromolecule transport may also be influenced by the SPS.[13]

Specific evaluations of the structural changes that characterize SPS and IPS, and their functional consequences in experimental models of kidney disease, or in patients with renal dysfunction, are not yet available. The difficulties in obtaining such quantitative evaluations derive from the heterogeneous nature of these three-dimensional structures. In addition, they can be visualized only using electron microscopy, both transmission electron microscopy and scanning electron microscopy (SEM), but their quantification is not easy because they are in the inner portion of the glomerular capillary tuft.[14]

ULTRASTRUCTURE OF THE GLOMERULAR CAPILLARY MEMBRANE

Endothelial Cell Layer

Glomerular endothelial cells are the most fenestrated in the circulation, with a pore area in peripheral zone that occupies from 20% to 50% of the cell surface.[15] The surface of endothelial cells has been considered to have negative electric charge due to the presence of electrical charges of glycoproteins, glycosaminoglycans, and membrane-associated proteoglycans (glycocalyx).[16] These negative charges are expected to act as an electrostatic barrier to the transmural passage of anionic circulating proteins, such as albumin. Thus even if endothelial fenestrae are much larger than albumin (about 60 nm in diameter as compared with a radius of 3.6 nm for albumin), negatively charged circulating macromolecules stay away from the endothelial surface due to electrical repulsion and remain within the circulation. It is now evident[1] that the first restriction to albumin filtration across the glomerular membrane consists of the endothelial surface layer and its role is to substantially decrease protein concentration in the fluid that enters the GBM layer. Endothelial cell glycocalyx expression is importantly affected by fluid shear stress.[17] Increased shear stress is associated with glycocalyx formation and reorganization on the cell surface in contact with fluid flow, with lower expression in static conditions. Thus pathologic conditions in which changes in glomerular capillary flow (i.e., reduced flow) may occur are expected to decrease glycocalyx formation and consequently reduce the retention of anionic proteins within the bloodstream. It has been demonstrated[18] that disruption of the endothelial glycocalyx increases glomerular albumin filtration even in the presence of only minor changes in both the GBM and glomerular epithelial cells. The role of the glomerular polysaccharide-rich endothelial surface layer (ESL) to act as a filtration barrier for large molecules such as albumin has been recently confirmed in C57Bl/6 mice given long-term infusion of hyaluronidase, a hyaluronan degradating enzyme that disrupts the endothelial glycocalyx proteoglycans.[19,20] A new electron microscopy technique that allows visualization of the ESL and albumin transport within the entire glomerular section at nanometer resolution was used in this set of experiments.[21] It was shown that glomerular fenestrae are filled with dense negatively charged polysaccharide structures that are largely removed in the presence of circulating hyaluronidase, leaving the polysaccharide surface of other glomerular cells intact.[19] Both retention of cationic ferritin in the glomerular basement membrane and systemic blood pressure were unaltered. Hyaluronidase treatment, however, induced albumin passage across the endothelium in 90% of glomeruli, whereas this could not be observed in untreated control animals. Nevertheless, there was no net albuminuria due to binding and uptake of filtered albumin by the podocytes and parietal epithelium. The ESL structure and function completely recovered after

cessation of hyaluronidase infusion. Thus the polyanionic ESL component hyaluronan is a key component of the glomerular endothelial permeability barrier whose reduction facilitates albumin passage across the endothelial layer and the GBM toward the epithelial compartment.

Glomerular Basement Membrane Organization

The basement membrane layer that characterizes the capillary wall (see Chapter 2) has been shown to exert an important contribution to protein retention by the capillary wall. The molecular composition and organization of this basement membrane would suggest a sieving function due to both size and charge.[22] Structural proteins such as collagen type IV and laminin, as well as heparansulfate proteoglycans, represent not only a steric hindrance but also a charge effect on the filtration of circulating molecules. Both vivo and in vitro studies[23] indicate that small neutral and charged solutes are freely filtered across this extracellular matrix layer, but an important restriction is observed for macromolecules the size of albumin or larger. Thus changes in composition and/or organization of GBM molecules are expected to reduce water filtration and retention of circulating macromolecules.[24] The role of GBM in glomerular permselectivity is highlighted by the discovery of mutations affecting genes encoding GBM components in humans and mouse models.[25] Mutations in the *COL4A3*, *COL4A4*, or *COL4A5* genes that encode collagen type IV α3, α4, and α5 chains, respectively, cause Alport syndrome, a hereditary glomerular, auditory, and ocular disease.[26] Mutations in the gene encoding laminin β2 (*LAMB2*) cause Pierson syndrome, a congenital nephrotic syndrome with associated extrarenal manifestation.[27] Studies using knockout mouse models of Alport and Pierson syndromes have documented that GBM, defective of these specific components, is more permeable to ferritin or albumin than is the normal GBM, indicating it has a role in glomerular permselectivity.[28,29]

Tight adherence to the basement membrane is required to prevent podocyte detachment into the Bowman capsule. On a molecular level, a multitude of adhesion receptors including heterodimeric integrins mediate interaction of cells with the surrounding basement membrane.[30,31] One common form of integrin-mediated adhesion is focal adhesions (FAs), which have been extensively studied in cultured cells.[31] Recently, FERM-domain protein EPB41L5 has been identified as a highly enriched podocyte-specific FA component.[32] This provided the clue to document that genetic deletion of the related *Epb41l5* gene resulted in severe proteinuria, detachment of podocytes, and development of focal and segmental glomerulosclerosis.[32]

Epithelial Filtration Slits

A large amount of experimental and clinical research has been generated in the past few decades on the molecular and structural composition of the epithelial junctional complex, known as filtration slits. The characterization of several molecular components of this structure[33] has allowed detailed definition of the proteins that compose the filtration slits (see Fig. 30.1); however, detailed information on the ultrastructure of this intracellular junction is still under investigation.[5] The original observations by Rodewald and Karnowsky[34] suggested a zipper-like structure of the epithelial filtration slit, with rectangular openings of 4 by 14 nm. These

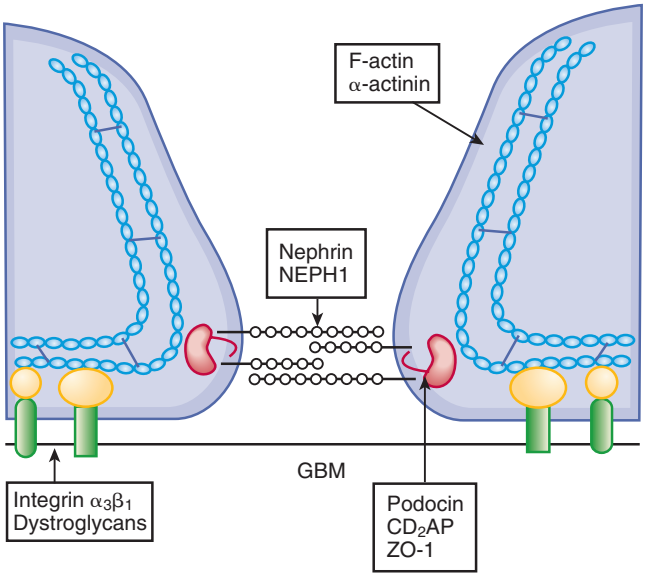


Fig. 30.1 Hypothetical model of the podocyte slit diaphragm. See text for discussion. (From Jalanko H. Pathogenesis of proteinuria: Lessons learned from nephrin and podocin. *Pediatr Nephrol.* 2003;18:487–491, with permission.)

dimensions are in contrast with the observation that a limited amount of albumin can traverse the filtration barrier in physiologic conditions,[1] because the mean molecular radius of albumin is 3.6 nm. Observations with high-resolution SEM and three-dimensional electron microscopy reconstruction[35] suggest that the filtration slits are perforated by larger openings of the size of albumin, with more complex geometry. The morphology of the filtration slit has been further imaged with high-resolution SEM, providing evidence of a new ultrastructure composed of circular pores of different sizes with an average radius of 12 nm[36,37] (Fig. 30.2). Despite the small size of filtration slit openings, a large amount of plasma water is filtered because of the high filtration slit length per unit surface area. As mentioned earlier, under physiologic conditions about 20% of peripheral capillary filtering surface is directly in communication with the Bowman space, and the epithelial slits are the last resistance encountered by water and filtered solutes.[11] In the remaining portion of the glomerular membrane, water and solutes, after passing through the filtration slits, must traverse the SPS and the IPS before arriving in the Bowman capsule.[11]

It should also be considered that the flow of ultrafiltrate over the cell surface directly causes shear stress (SS) on the podocyte membrane associated with the filtration slit as well as to cell body. In animal models of solitary kidney where filtrate flow is increased, SS on the podocyte surface increases 1.5- to 2-fold.[38] These forces are highest within the central portion of the filtration slit diaphragm. The forces acting parallel to the GBM are balanced by the mechanical resistance produced by the slit diaphragm complex, opposing foot processes, preventing widening of the slit.[39] On the other side, SS acting perpendicular to the GBM plane, tends to detach the foot process from the GBM. However, these shear forces are balanced by the tight junctions between podocyte cell membrane and GBM proteins that allow transmission of this mechanical load into the cytoskeleton apparatus. These

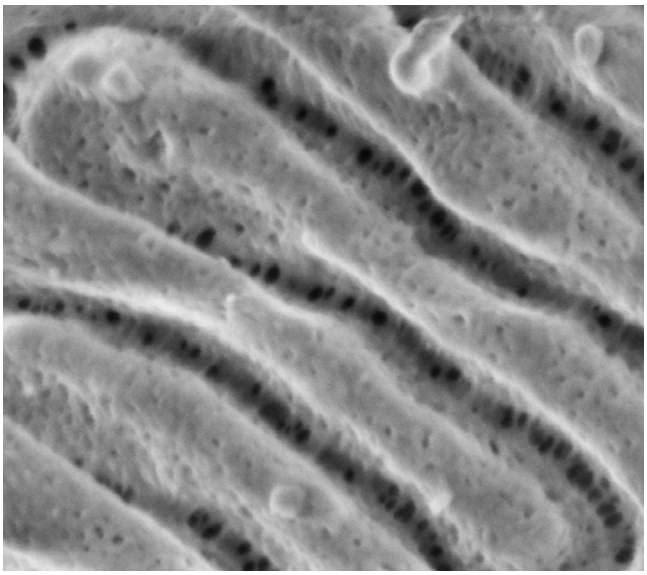


Fig. 30.2 Visualization of epithelial filtration slits obtained using scanning electron microscopy and an in-lens detector to enhance electron detection. Sample was obtained from a Wistar rat, dehydrated with a critical point dryer. The ultrastucture of the filtration slit appears different from the model conventionally proposed in the literature.[34] The radius of the circular pores averages 12 nm. (From Gagliardini E, Conti S, Benigni A, et al. Imaging of the porous ultrastructure of the glomerular epithelial filtration slit. *J Am Soc Nephrol.* 2010;21:2081–2089.)

complex mechanical challenges present in physiologic conditions within the podocyte, induced by glomerular ultrafiltrate flow, make mechanotransduction an important function of podocytes. In addition, SS acting on the podocyte membrane adjacent to the filtration slit, as well as to the entire cell body, seems to play a central role in the process of podocyte damage that results in foot process effacement as well as in podocyte detachment from the GBM.[39]

THEORETICAL MODELS OF GLOMERULAR PERMSELECTIVITY

In addition to structural investigation, functional evaluations of the glomerular capillary wall permselectivity have been extensively used to characterize physiologic conditions and to quantify the effects of pathologic changes. These studies are based on the estimation of filtration of endogenous plasma molecules, such as albumin, IgG, and other proteins, or on the use of test macromolecules of different size, either neutral or electrically charged. Macromolecule filtration depends on convective and diffusive transport that is influenced by glomerular hemodynamic conditions (glomerular plasma flow and pressure) and water filtration. As described later, several investigators have developed theoretical models to derive intrinsic sieving properties of the capillary wall from estimation of macromolecule filtration in experimental and human studies.

Heteroporous Models of Glomerular Size Selectivity

The most-used theoretical models of glomerular size-selective function are based on the assumption of water-filled pores of different sizes as functional equivalents of the glomerular membrane. The passage of water is calculated along the network taking into account the balance between hydraulic and oncotic pressure, as well as membrane hydraulic permeability.[9,40] For the calculation of solute filtration, convective and diffusive transport are taken into account, whereas pore resistance to solute filtration is based on steric and hydrodynamic hindrance.[10,40] The use of these models indicated that glomerular hypothetical pores have mean radius of 4.5–5.0 nm in humans, and a lognormal statistical distribution of pore size around the mean. However, the best simulation of experimental measurements has been obtained assuming that in parallel to restrictive pores, there is a nonselective shunt pathway.[41]

The application of these theoretical models clearly showed that in several proteinuric conditions the increased glomerular filtration of the largest neutral test macromolecules is not associated with important changes in the size of restrictive pores, but rather with changes in the nonselective shunt pathway.[42] This suggests that in normal conditions the small amount of albumin present in the urine may be the result of a small amount of protein filtration that takes place in some focal areas of the epithelial junction, while the majority of the filtration slits retain the protein.

Fiber Models of Glomerular Size Selectivity

Fiber models of solute filtration across the glomerular membrane have also been developed and used.[1] Similar to porous models, the fiber models allow separation of the effect of glomerular hemodynamic changes from those related to intrinsic changes of glomerular membrane selective properties. The advantage of the fiber model is that other than steric hindrance, the effect of membrane and protein electrical charge can be embedded in the model, allowing the estimation of changes in membrane properties both in terms of molecular structural organization and electric charge.[43] This modeling approach indicates that filtration of albumin is importantly affected by electrical charge, whereas on the basis of size selectivity alone the molecule could easily escape the capillary membrane.

Multilayer Membrane Models. The structural complexity of the glomerular capillary wall suggested a need to develop more complex theoretical models to simulate more reliably the resistance to water and solute movement across the membrane. These models have been developed and tested by Deen et al.,[22] with the aim to estimate the role of individual layers on the filtration of water and solutes. In these models the resistance of endothelial cells, GBM, and epithelial cells is assumed to act in series. In normal conditions, hydraulic resistance of the endothelial layer is negligible, while GBM and epithelial resistance are comparable. The contribution of the three layers to solute hindrance has been considered and the major contribution to membrane selectivity is exerted by the filtration slit.[44] Although these models describe in detail the physical interaction of water and macromolecules with the membrane structure, their application is difficult because they require extensive measurement of utrastructural parameters.

Models of Glomerular Charge Selectivity

As mentioned previously, the fact that negative electrical charges are present in the glomerular membrane (in the glycocalyx of endothelial cells, the negatively charged heparansulfate of the GBM and the glycoproteins of the cell membrane of podocytes) strongly suggests that circulating negatively charged proteins, like albumin, are restricted within

the circulation not only for their size but also for electrical charges. The use of theoretical models for the simulation of charge-selective function of the glomerular membrane allowed estimation of the amount of electrical charge present within the membrane.[10] These studies indicated that electrical charge is an important component of glomerular permselective function, and changes in membrane electrical charge can explain abnormal albumin filtration even without changes in membrane structural parameters, such as pore size or fiber size and length per unit volume. However, the use of these models to investigate glomerular membrane charge is limited by the difficulties in measuring glomerular filtration of charged test solutes that interfere with circulating macromolecules and are not filtered simply on the basis to their molecular shape and electric charge alone.[45]

PROTEIN REABSORPTION BY THE PROXIMAL TUBULE

PROXIMAL TUBULE STRUCTURE AND FUNCTION

The glomerular ultrafiltrate, once flowing inside the proximal tubule, undergoes important changes in composition, due to processes of water and solute reabsorption. In addition to small solutes and electrolytes, proteins such as albumin are also reabsorbed.[46] Thus final urinary excretion of proteins depends largely on the interaction of proteins with proximal tubular epithelial cells. These cells form a compact epithelial layer with a basal side in contact with the tubular basement membrane, an intercellular junction, and a luminal surface in contact with tubular fluid. They are characterized by a large number of mitochondria, an index of important metabolic activity, and a prominent layer of microvilli (Fig. 30.3) that results in the extension of the luminal cell surface area.

The microvillar membrane is the site for receptor mediated endocytosis of low-density lipoprotein and negatively charged proteins. Albumin in the proximal tubule undergoes specific binding to the extracellular domain of a membrane receptor complex, megalin–cubilin receptor[46] (Fig. 30.4), which is followed by the internalization of the protein by membrane vesicles. These vesicles are then processed for protein degradation, amino acid transport to basal membrane of tubule cells, release into interstitial space, and ultimately into peritubular capillaries. Receptors are recycled into the luminal cell membrane by dense apical tubules.

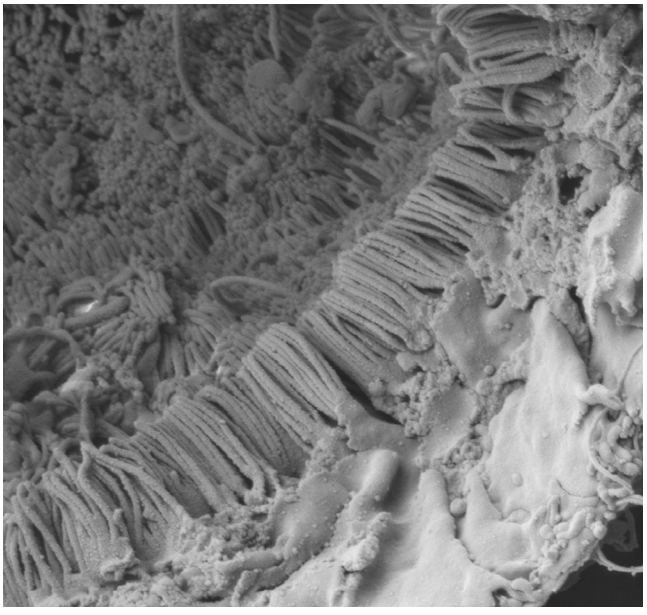


Fig. 30.3 Scanning electron microscopy of proximal tubule cells. Inner cell membrane is covered by microvilli of the brush border. In order to be taken up by cell receptors, albumin must diffuse through the dense layer of microvilli.

The amount of albumin filtered at glomerular level and that reabsorbed by proximal tubule cells is not easy to quantify. Ideally one would have to sample the early proximal tubule and quantify albumin concentration in these microsamples. Despite technical difficulties, micropuncture techniques have been used to avoid sample contamination with plasma present close to the puncture site (in interstitial space and peritubular capillaries). The protein concentration in the urinary space was estimated to range from 10 to 25 μg/mL.[47,48] More recently, direct in vivo imaging of fluorescent albumin by two-photon microscopy has been used to directly estimate albumin concentration in the Bowman capsule fluid.[49] It has been recently demonstrated that reliable measurements of albumin fractional clearance, the ratio between urinary space and plasma albumin concentration, allow estimation of an albumin concentration of about 60 μg/mL in the Bowman capsule in normal conditions in the rat,[50] corresponding to a fractional clearance of 0.002. Once filtered at glomerular level, albumin and smaller proteins are almost entirely reabsorbed at the proximal tubular level. In pathologic conditions, when the filtered load overwhelms the reabsorptive capacity, proteins are detected in the urine.

THEORETICAL MODELS FOR TUBULAR REABSORPTION

The process of albumin reabsorption by proximal tubule cells has been modeled[51] to allow a quantitative assessment of the relationship between albumin ultrafiltration at the glomerular level, proximal tubular uptake, and final excretion in the urine. In this model, the process of diffusion of albumin across the microvillar space and the uptake of the protein by tubule cell receptors are taken into consideration. The amount of albumin that is reabsorbed during the proximal tubule passage is simulated assuming the presence of a high-affinity site for binding and internalization of albumin at the base of tubule cell microvilli. According to in vitro and ex vivo data, these receptors are assumed to be half-saturated at concentrations similar to those mentioned for albumin in the Bowman capsule (20–30 μg/mL). The effect of the assumption of different values for the maximum absorptive capacity (V_{max}) on the albumin concentration along the proximal tubule is reported in Fig. 30.5. This modeling approach showed that the transport of albumin across the microvillar space has a modest effect on the value of V_{max} needed to fit micropuncture data.

The two most important parameters that determine the fraction of reabsorption of albumin are the single-nephron glomerular filtration rate (SNGFR) and the albumin concentration in the filtrate fluid (Cb). A 50% increase in SNGFR is predicted to cause a four- to fivefold increase in albumin excretion in rats and humans. For large increases in Cb, such as those measured by micropuncture,[52] there is a threshold above which the reabsorption of albumin is overwhelmed and the protein appears in the urine. According to theoretical analysis[51] this value corresponds to an albumin

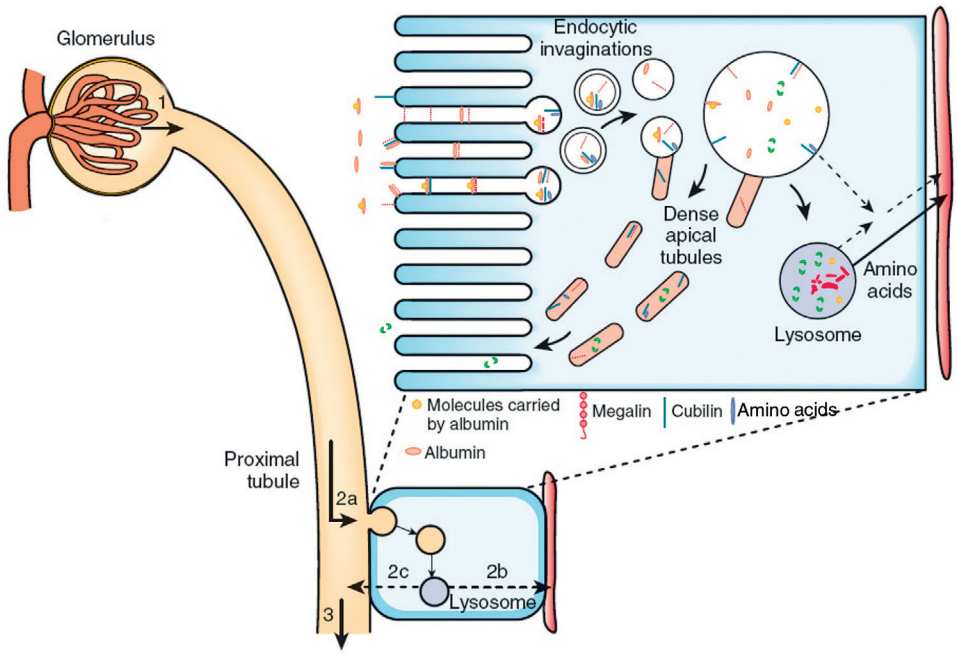


Fig. 30.4 Pathways of albumin degradation in the proximal tubule. Albumin is filtered in the glomeruli *(1)* and reabsorbed by the proximal tubule cells by receptor-mediated endocytosis *(2a)*. Internalization by endocytosis is followed by transport into lysosomes for degradation. Some intact albumin may escape tubular reabsorption *(3)*, the amount being greater as the glomerular filtration fraction of albumin increases or tubular function is compromised. The *upper right* shows a schematic representation of the intracellular pathways following endocytic uptake of albumin and possible associated substances. Following binding to the receptors, cubilin, or megalin, the receptor–albumin complex is directed into coated pits for endocytosis. The complex dissociates following vesicular acidification, most likely also leading to the release of any bound substances. Albumin is transferred to the lysosomal compartment for degradation. Some albumin may be degraded within a late endocytic compartment and recycled as fragments to be released at the luminal surface. Alternatively, albumin fragments may be recycled from the lysosomal compartment by a yet unknown route. Receptors recycle through dense apical tubules, whereas released substances carried by albumin may be released into the cytosol or transported across the tubular cell. (From Birn H, Christensen EI. Renal albumin absorption in physiology and pathology. *Kidney Int.* 2006;69:440–449, with permission.)

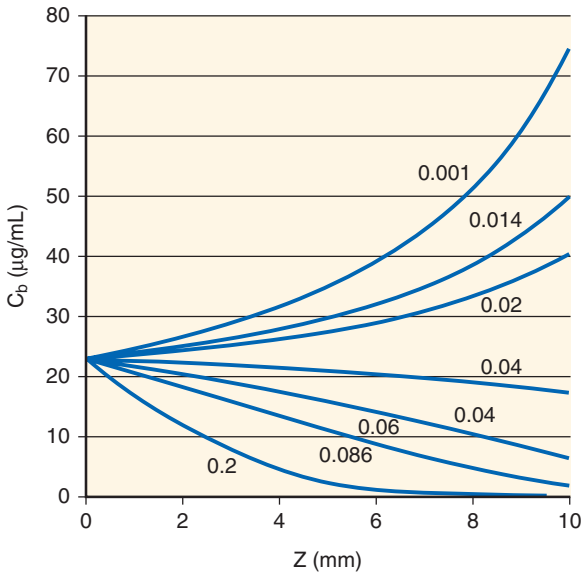


Fig. 30.5 Theoretical calculation of albumin concentration along the proximal tubule. Predictions of bulk albumin concentration (*Cb*) vs. axial position (*z*) in rats for V_{max} ranging from 0.001 to 0.2 ng/sec per millimeter squared. The curve that most closely corresponds to normal rats is that for V_{max} = 0.086 ng/sec per millimeter squared. (From Lazzara MJ, Deen WM. Model of albumin reabsorption in the proximal tubule. *Am J Physiol Renal Physiol.* 2007;292:F430–439, with permission.)

fractional clearance of approximately 0.001. The combination of increased SNGFR and elevation in filtrate albumin concentration is shown to have important additive effects.

PROTEINURIA OF GLOMERULAR ORIGIN

Changes in glomerular protein filtration and/or defects in tubular reabsorption cause the appearance of proteins in the urine. At values exceeding 300 mg/day, or 200 mg/L the condition is termed *proteinuria*. Smaller amounts of proteins may appear in the urine in the early stages of progressive diseases, such as diabetic nephropathy. In this case an albumin excretion between 30 and 300 mg/day (20–200 mg/L) is termed *microalbuminuria*.[53] Proteinuria is considered severe or in the "nephrotic range" when protein excretion is greater than 3.5 g/day.[1] When proteins in the urine have a large molecular weight, they are considered to have glomerular origin. If the molecular weight is low, there is evidence that the defect causing proteinuria is likely related to abnormal proximal tubular reabsorption, often related to toxic damage of tubule cells.[54] Proteinuria associated with progressive kidney disease is predominantly of glomerular origin and mainly composed of plasma albumin. Mechanisms responsible for glomerular proteinuria are discussed in the next section.

GLOMERULAR PERMSELECTIVE DYSFUNCTION

As mentioned earlier, abnormal plasma protein filtration at glomerular level may be caused by a defect in both size and

charge selectivity. Glomerular size-selective dysfunction has been extensively investigated in several kidney diseases using neutral test macromolecules, usually neutral dextrans.[42,55] These studies demonstrated that in most cases there are statistically significant changes in fractional clearance of the largest test macromolecules, whereas the fractional clearance (sieving coefficient) of molecules the size of albumin is unaltered. These data consistently indicate that permselectivity defects responsible for albumin filtration must be focal and likely due to changes in glomerular cell components, most likely the podocytes. These proteinuric conditions are frequently associated with podocyte foot process effacement and simplification, and likely with defective intercellular junctions.[56]

The quantification of the contribution of charge-selectivity defects to proteinuria is more difficult. A few experimental and clinical investigations clearly indicate that proteinuria is indeed associated with abnormal filtration of charged macromolecules,[57,58] but these data have been questioned because electrically charged test or endogenous macromolecules in the circulation are expected to interfere with other circulating charged solutes and cell membranes.[1] This would result in measured fractional clearance that does not represent effective transport of probe macromolecules. However, even without direct evidence that glomerular membrane charge distribution is altered in proteinuric conditions, the evidence that glomerular albumin filtration is increased without important changes in the fractional clearance of test macromolecules of the same size strongly suggests a role for a charge-selectivity defect in proteinuria of glomerular origin.[42,58]

Experimental and clinical research has allowed the identification of molecular defects underlying some genetic disorders associated with nephrotic syndrome. In Finnish-type nephropathy a defect in the nephrin gene NPHS1 is responsible for glomerular dysfunction, proteinuria, and end-stage renal disease.[3,59] Similarly, defects in other genes (NPHS2, LMX1B and several others) have been shown to result in defective structure and function of filtration slit proteins or glomerular epithelial cells.[60]

Another condition in which proteinuria is manifest is ischemia–reperfusion injury.[61] Studies in kidney transplantation and in experimental settings suggest that abnormal elevation of protein in the urine in this condition occurs without major changes in glomerular capillary membrane structure. The evidence indicates[62] that ischemia per se is responsible for loss of glomerular endothelial glycocalyx, and the previously mentioned effect of fluid shear stress on endothelial cell glycocalyx would reinforce this evidence. Thus, abnormal elevation of glomerular protein filtration may derive from selective changes in ultrastructure and function of membrane components.

TUBULAR HANDLING OF EXCESSIVE FILTERED PROTEINS

EFFECTS OF PROTEIN FILTRATION ON PROXIMAL TUBULE CELLS

An excessive increase in albumin and other plasma protein filtration may result from a defective glomerular capillary membrane and/or an increase in SNGFR. Both conditions, and their combination, result in elevated protein concentration in the ultrafiltrate. These filtered proteins are expected not to be entirely reabsorbed by proximal tubule cells, because protein reabsorption is believed to operate near maximum under physiologic conditions.

The presence of high protein concentration within the renal tubule may influence the progression of disease processes. There are at least two phenomena that are expected to occur. The first is related to the fact that if albumin is still present in tubular fluid at the end of the proximal tubule, its concentration increases substantially along the remaining portion of the nephron because of water reabsorption. Thus protein concentration in the distal tubule and collecting duct can reach very high values even for a small amount of proteins filtered at glomerular level, with the possibility for these proteins to precipitate and form protein casts.[63] Tubular obstruction may then occur and the entire nephron function lost, with complete loss of glomerular water filtration.

In addition, structural changes are expected to occur with tubular atrophy, disconnection of the tubule from the Bowman capsule and glomerular capillary tuft structural changes. This condition is frequently observed in proteinuric kidney diseases at experimental and clinical level.[64]

Even before tubular obstruction, important functional changes are expected to occur in proximal tubule cells exposed to abnormal protein concentrations. The protein overload of these cells exposes them to increased workload and this can lead to loss of reabsorptive capacity due to loss of receptor activity.[65] In this condition, the elevated protein concentration along the proximal tubule further increases due to the lower level of absorption and the concomitant water reabsorption. Thus a vicious circle develops, inducing further damage in tubule cells and progressively higher protein concentration along the entire nephron. The consequences of this abnormal glomerular filtration of plasma proteins at both the organ and systemic level are discussed in the following sections.

RENAL CONSEQUENCES OF PROTEINURIA

GLOMERULAR DAMAGE

Data from animal models have shown that a wide variety of insults result in a common pathway of glomerular capillary hypertension, increased permeability with excess passage of proteins across the glomerular capillary wall, and progressive glomerular injury[66] (Fig. 30.6). The key glomerular lesion is sclerosis, characterized by accumulation of extracellular matrix and obliteration of the capillary tuft leading to the loss of renal function.

PODOCYTES: CHANGES IN FUNCTION AND CELL NUMBER

Podocytes show a fairly uniform pattern of response to damage. The intercellular junction and cytoskeletal structure of the foot processes are altered, and the cell shows a simplified, effaced phenotype.[67,68] These alterations result in the disappearance of the typical slit diaphragm structures and the development of proteinuria. Although podocyte effacement is a hallmark of podocyte disease and nephrotic syndrome, damage to these cells may present as very subtle changes that are difficult to quantify.[69] Major advances in the field of live imaging have allowed the investigation of

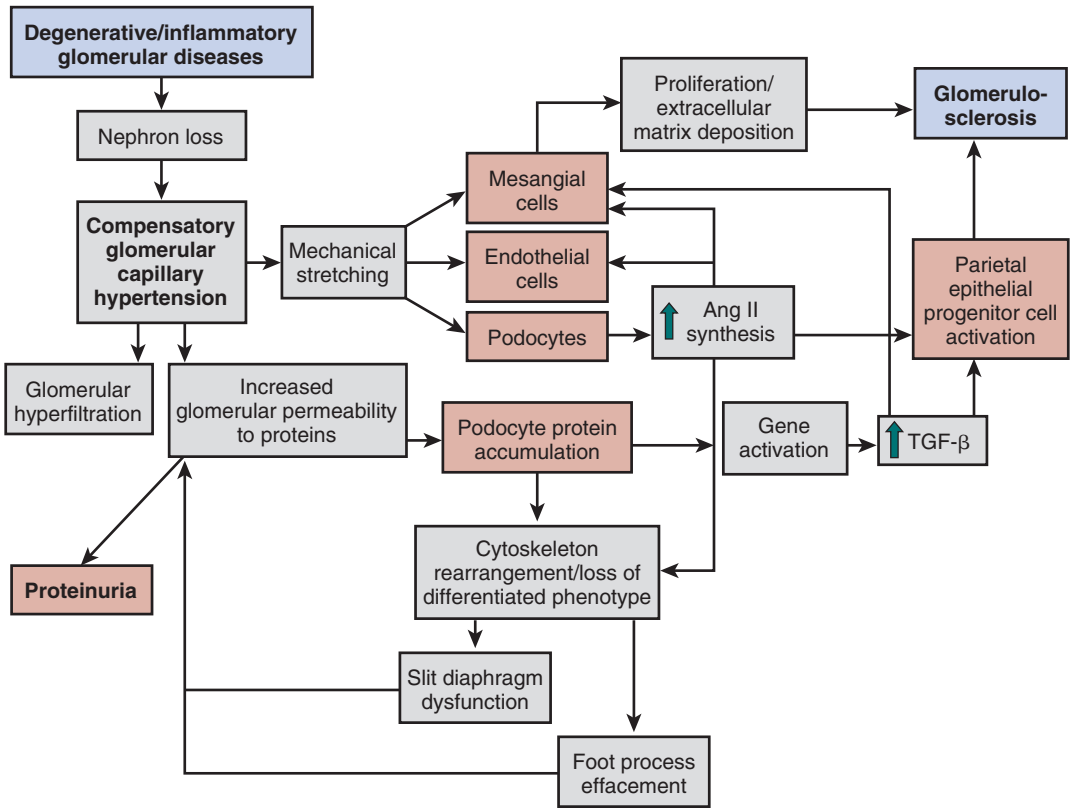


Fig. 30.6 Mechanisms of progressive glomerular injury. A reduction in the number of nephrons as a consequence of various glomerular diseases results in compensatory glomerular hemodynamic changes that are ultimately detrimental. In particular, by mechanical stretching, the increased glomerular capillary pressure directly injures glomerular cells. Glomerular hypertension also impairs the glomerular capillary size–selective function, which causes excessive protein ultrafiltration and eventually podocyte injury and proteinuria.

podocyte biology in unprecedented detail.[70-72] These new tools are likely to facilitate the next stage in gaining insights to podocyte responses to injury. So far there is evidence that experimental models of chronic proteinuria as well as their human counterparts (that is, minimal change glomerulopathy, focal and segmental sclerosis, diabetic nephropathy, and membranous nephropathy) have in common ultrastructural findings of severe glomerular epithelial cell damage that include vacuolization, fusion of foot processes and focal detachment of epithelial cells from the underlying basement membrane.[73] These changes appear to be the consequence mainly of persistent abnormalities in intraglomerular capillary hemodynamics. Increased capillary hydraulic pressure and flow and activation of local tissue renin–angiotensin system in podocytes[74] eventually impairs the size-selective function of the glomerular capillary wall, allowing excess plasma protein to move into the urinary space.[75]

In addition to being affected by mechanical stress, podocytes are also damaged by excessive protein load resulting from alterations of glomerular permeability to macromolecules. Protein uptake by podocytes may occur through binding to megalin, a receptor for albumin and immunoglobulin (Ig) light chains, which is endocytosed after ligand binding, as shown in cultured murine podocytes.[76] Mice with protein overload proteinuria induced by repeated injection of bovine serum albumin developed podocyte injury followed by glomerulosclerosis.[77-79] Evidence of a causal link between podocyte protein overload and podocyte damage is provided by studies showing that in rats with renal mass reduction protein accumulation in podocytes preceded cell dedifferentiation and injury, as characterized by loss of synaptopodin and an increase in desmin expression.[80]

Podocyte abnormalities were accompanied by upregulation of transforming growth factor-β (TGF-β) messenger ribonucleic acid (mRNA) and enhanced production of the related protein.[80] In vitro, albumin loading of immortalized mouse podocytes promoted actin–cytoskeleton rearrangement and upregulation of intracellular transduction signals, such as activating protein-1 (AP-1), which is a known stimulus of TGF-β1 synthesis.[81]

Podocytes possess a complex contractile structure composed of F-actin microfilaments, most abundant in the foot process, connected with adaptor molecules that anchor the slit diaphragm proteins and α3β1 integrins, transmembrane proteins that form focal adhesion complexes and mediate podocyte–GBM matrix interaction.[82,83] In vitro, actin filament disorganization, as occurs after albumin loading of mouse podocytes,[81] is closely associated with podocyte shape changes that affect cell adhesion to the extracellular matrix. Podocyte detachment from the GBM likely underlies the decrease in podocyte number in proteinuric glomerular diseases, which has been shown in many experimental and clinical studies.[84,85]

Apoptosis is considered an additional cause of podocyte loss in proteinuric glomerulopathies. Once detached from the GBM, podocytes become extremely susceptible to apoptosis.[67] Furthermore, apoptosis may be promoted by locally

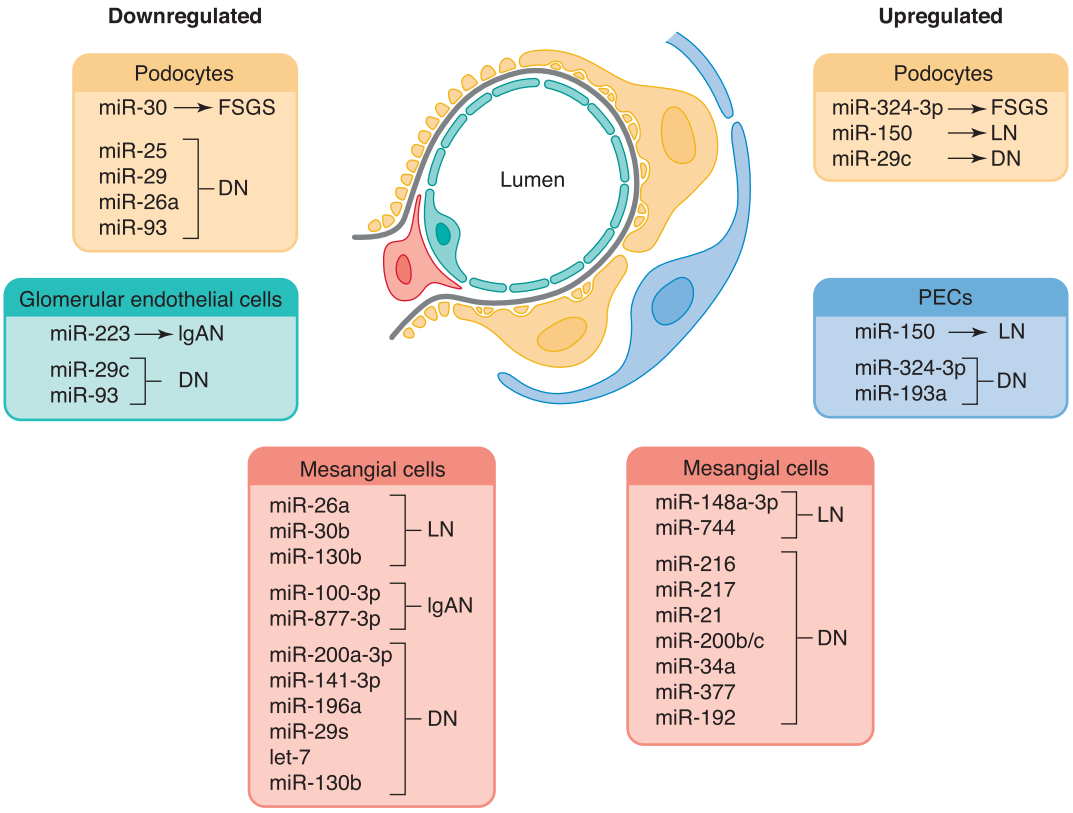


Fig. 30.7 MicroRNA (miRNA) dysregulation in glomerular disease. Changes in miRNAs in different populations of glomerular cells (podocytes, parietal epithelial cells, glomerular endothelial cells, and mesangial cells) occurring in focal and segmental glomerulosclerosis *(FSGS)*, lupus nephritis, IgA nephropathy, and diabetic nephropathy. *DN*, Diabetic nephropathy; *IgAN*, IgA nephropathy. (From Trionfini P, Benigni A. MicroRNAs as master regulators of glomerular function in health and disease. *J Am Soc Nephrol.* 2017;28:1686–1696.)

produced proapoptotic factors. Studies have demonstrated that exogenous TGF-β1 induced apoptosis in cultured podocytes via the p38 mitogen-activated protein kinase (MAPK) and classic caspase-3 pathways.[86] This effect occurred only in wild-type, not in p21-null cultured podocytes, indicating that the cyclin-dependent kinase (CDK) inhibitor p21 is required for TGF-β1–induced apoptosis.[87] Of note, like TGF-β1, p21 is increased in podocytes in experimental models of membranous nephropathy[88] and diabetic nephropathy.[89] In summary, protein accumulation in podocytes induces TGF-β1 production, leading to podocyte apoptosis.

Recent evidence indicates that angiotensin II (Ang II) contributes to perpetuate podocyte injury in proteinuric nephropathies eventually promoting progression to end-stage kidney disease.[90] Mechanical strain increases Ang II production and expression of Ang II type 1 (AT_1) receptors in podocytes[74] potentially contributing to further sustaining the glomerular hypertension-induced damage in chronic kidney disease. However, there is also evidence that, independent of its hemodynamic effect, Ang II may directly impair the glomerular barrier sieving function, possibly through inhibition of nephrin expression by podocyte, the essential protein component of the glomerular slit diaphragm.[91,92] This observation has been confirmed in studies in diabetic animals showing that blockade of Ang II synthesis/activity preserved the expression of nephrin in the glomeruli and prevented overt proteinuria.[93,94] Thus at least in diabetes, a pathogenetic relationship between Ang II and early proteinuria via functional podocyte alteration through modulation of nephrin protein level has been suggested. Moreover, in the setting of diabetes, after the initial insult of hyperglycemia and intraglomerular hypertension, Ang II plays a relevant role to sustain glomerular injury via persistent activation of Notch1 and Snail signaling in the podocyte, eventually resulting in persistent downregulation of nephrin expression.[95] The consistency of these findings in diabetic ZDF rats with overt nephropathy and in type 2 diabetic patients with established nephropathy provides robust insight to implicate an important role for the Ang II Notch1/Snail axis in perpetuating podocyte damage.

MicroRNAs (miRNAs) are a class of short (21–24 nucleotides) noncoding RNAs that regulate gene expression through posttranslational and epigenetic mechanisms, thereby affecting several cellular processes from development to disease conditions.[96] miRNAs are critical players in podocyte homeostasis (Fig. 30.7), because targeted deletion of the helicase with RNAse motif Dicer or the class II ribonuclease III enzyme Drosha in these cells leads to proteinuria and glomerulosclerosis.[97-100] Moreover, several miRNAs have been found to be dysregulated in podocyte injury, a pathologic mechanism causing glomerular injury and sclerosis. Because mature podocytes must withstand fluctuating pressures and potentially harmful molecules contained in the primary filtrate, they are unlikely to be static structures.[101] Remodeling of the actin cytoskeleton plays a primary role in the structural adaptations made by podocytes to preserve their

glomerular filtration properties. The podocyte cytoskeleton is finely regulated by a set of miRNAs that are expressed mainly in the adult kidney, such as miR-30, miR-132, miR-134, and miR-29a. miR-30 family members are highly expressed in human podocytes and, in addition to protecting them against apoptosis,[102] they promote podocyte actin fiber stability by controlling calcium/calcineurin signaling through the inhibition of several components of this pathway.[103,104] Dysregulation of calcium/calcineurin signaling leads to podocyte cytoskeletal damage, which is a key feature of various glomerular diseases, such as FSGS, characterized by the early onset of podocyte injury.[103] It is notable that miR-30s are significantly downregulated in the podocytes of patients with FSGS and in the rat model of FSGS induced by the podocyte toxin puromycin aminonucleoside. Furthermore, fibrogenic factors, such as TGF-β, reduce miR-30 expression both in vivo and in cultured podocytes.[102,105] A recent study underlines the role of miRNAs in regulating podocyte cytoskeletal dynamics. The study showed that brain-derived neurotrophic factor can repair podocyte damage both in vitro and in a mouse model of FSGS by inducing miR-132 and inhibiting miR-134 expression upon binding to its receptor on podocytes. Brain-derived neurotrophic factor–induced modulation of miR-132 and miR-134 has been found to be essential for increasing actin polymerization, favoring foot process elongation that contrasts the cell flattening induced by proteinuric conditions.[106]

A crosstalk with proximal tubule cells has been recently suggested to contribute to podocyte function through the release of nicotinamide mononucleotide (NMN) that might have implications for persistent podocyte dysfunction in proteinuric diseases.[107] In a mouse model, diabetes induced downregulation of Sirtuin1 (Sirt1), a highly conserved protein deacetylase in proximal tubules.[108] The low Sirt1 expression reduces the release of nicotinamide mononucleotide by tubule cells, eventually decreasing local NMN concentrations. In vitro studies have shown that in the absence of NMN the expression of the tight junction protein claudin-1 in podocytes is no longer silenced.[107] Claudin-1 is reported to activate the intracellular β-catenin–Snail pathway[109] that eventually leads to glomerular barrier dysfunction through downregulating synaptopodin or podocin expression in podocytes.[110]

MESANGIAL CELLS: PROLIFERATION AND DEPOSITION OF EXTRACELLULAR MATRIX

Because it is close to the capillary lumen, the mesangium may be exposed to macromolecules crossing the endothelial layer, although under normal conditions they do not accumulate.[111] However, in rats having undergone unilateral nephrectomy[112] or in puromycin-aminoglycoside–induced nephrosis,[113] intravenous infusion of colloidal carbon leads to the accumulation of the macromolecular tracer in the mesangial space. To prevent accumulation of proteins, mechanisms exist for their effective removal. These include transport along the mesangial stalk in cleft-like spaces as well as phagocytosis and degradation by mesangial cells.[114]

It has been shown that IgG and IgA can be taken up by both receptor-independent and receptor-mediated processes.[115] Another important factor for clearance of Igs from the mesangium may be complement factor D, a serine protease essential for activation of the complement system through the alternative pathway, which is constitutively expressed within the glomerulus.[116] Interestingly, mice deficient in complement factor D spontaneously develop a mesangial immune-complex deposition disease associated with albuminuria.[117] This indicates that complement factor D is necessary to prevent mesangial accumulation of immunoglobulin deposits.

Whether abnormal local accumulation of proteins promotes mesangial cell proliferation and mesangial matrix deposition remains, however, ill-defined. Nevertheless, the significance of the protein-clearing function of the mesangial cells has been illustrated by the deleterious consequences of mesangial immune complex accumulation leading to complement activation and generation of mediators of inflammation, such as reactive oxygen species, prostanoids, and cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6).[118]

The mesangial cell is a critical part of the glomerular functional unit interacting closely with endothelial cells and podocytes.[116] Alterations in one cell type can produce changes in the others. As such, key survival factors for mesangial cells, including platelet-derived growth factor-B (PDGF-B) are generated by endothelial cells, and mesangiolysis has been shown in knockout mice lacking endothelial PDGF-B.[116] Whether cytokines generated by podocytes also influence mesangial cells has yet to be clearly defined, but the observation that podocyte injury frequently results in mesangial cell proliferation supports the existence of such cytokine crosstalk.[116]

Besides PDGF-B, other growth factors shown to influence mesangial cell proliferation and mesangial matrix accumulation include PDGF-C, fibroblast growth factor, hepatocyte growth factor, epidermal growth factor (EGF), connective tissue growth factor, and TGF-β.[116] Effects of vasoactive hormones such as Ang II on mesangial cell proliferation may be mediated indirectly through the generation of growth factors such as EGF.[119] In rats with renal mass ablation, glomerular TGF-β1 upregulation is associated with phenotypic transformation of mesangial cells.[80] In vitro exposure of cultured murine mesangial cells to TGF-β induced a sclerosing phenotype as shown by α-smooth muscle actin (SMA) expression, which was blocked by anti-TGF-β1 antibodies.[80] Moreover, the transfection of the TGF-β1 gene into normal kidneys in rats or the transgenic TGF-β expression in mice increased extracellular matrix accumulation in the mesangial space.[120] At least in experimental diabetes, increased matrix production in mesangial cells is induced by a fine balance between the upregulation of miR-192, miR-200b/c, miR-216a, and miR-377 and the downregulation of miR-29s and let-7. High glucose, via TGF-β, upregulates miR-377, which suppresses the expression of p21-activated kinase and superoxide dismutases, leading to enhanced susceptibility to oxidant stress and the accumulation of fibronectin.[121] In mesangial cells, TGF-β lowers the expression of miR-29s[122] and let-7,[123] antifibrotic miRNAs targeting different isoforms of collagen. Moreover, decreased miR-29a expression attenuates the Dickkopf-1/Wnt/β-catenin signaling, contributing to apoptosis and extracellular matrix deposition in streptozotocin-treated mice.[124] Notably, in mesangial cells TGF-β induces signaling loops that amplify and create a chronic state of profibrotic pathway activation, modulating the expression of miR-192, miR-200s, miR-21, and miR-130b.[125-127]

ENDOTHELIAL CELLS: APOPTOSIS

Glomerular endothelial injury is a common feature of many human diseases, such as diabetic nephropathy, hypertension,

thrombotic microangiopathy and preeclampsia. Evidence has been provided that there is close crosstalk of podocytes with glomerular endothelial cells, a key interaction for the normal function of the glomerular capillary barrier.[128] The final steps in glomerular endothelial cell differentiation involve the formation of fenestrae, plasma membrane-lined circular pores that perforate the flattened glomerular endothelium.[129] The fenestrated phenotype of glomerular endothelial cells is induced by vascular endothelial growth factor (VEGF), a molecule constitutively expressed and secreted by podocytes.[128,129] That VEGF regulates fenestrae formation was suggested by the observation that mature fenestrated endothelium is typically located adjacent to podocytes expressing high levels of VEGF mRNA.[130] There is also in vitro evidence that VEGF type A and C regulate glycosaminoglycan synthesis, charge, and shedding of glomerular microvascular endothelial cell glycocalyx.[131] Moreover, deletion of the podocyte-specific transcription factor LMX1B in mice, which results in the loss of many features of podocyte differentiation, including VEGF-A expression, is associated with failure of glomerular endothelium to differentiate and develop fenestrae.[132] Thus, loss of podocytes secondary to protein-induced cell injury may lead to reduced VEGF production influencing glomerular endothelial fenestrae formation and eventually leading to endothelial cell apoptosis.[133] However, how VEGF reaches endothelial cells against the urine flow is not yet known. Conversely, recent in vitro evidence has shown that blockade of VEGF in glomerular endothelial cells enhanced the release of endothelin-1 (ET-1), which induced nephrin shedding from podocytes,[134] leading to further glomerular protein permeability dysfunction.

Moreover, endothelin-1 activates podocytes to release heparanase.[135] In mice, podocyte-specific deletion of the endothelin receptor prevented the diabetes-induced increase in glomerular heparanase expression and consequent reduction in heparan sulfate expression, endothelial glycocalyx thickness, and development of proteinuria that was observed in wild-type mice.[135] Heparanase-deficient mice were resistant to the development of proteinuria and renal damage upon induction of type 1 diabetes mellitus.[136] In addition, proteinuria was also reduced and renal function improved by treatment with the experimental heparanase inhibitor SST0001.[136]

Taken together, these studies indicate that the toxic effects of excess ultrafiltered plasma proteins on podocytes may alter podocyte–endothelial interaction, thereby further enhancing glomerular permeability to proteins through a complex interplay of molecular signaling.

PARIETAL EPITHELIAL CELLS: ACTIVATION

Changes in glomerular permselective function, as it occurs in proteinuric glomerulopathies, elevate the filtered load of plasma albumin and consequently its concentration in the Bowman space (Fig. 30.8). Evidence has been provided that the abnormally filtered albumin impairs the mechanism underlying regeneration of protein-induced damage to podocytes.[137] Glomerular injury caused by multiple etiologies can lead to activation and accumulation of parietal epithelial cells (PECs) within the Bowman space as a common response to damage,[138] as shown in several human proliferative glomerulonephritides. Although extracapillary proliferation is a relatively straight forward pathologic change to recognize,

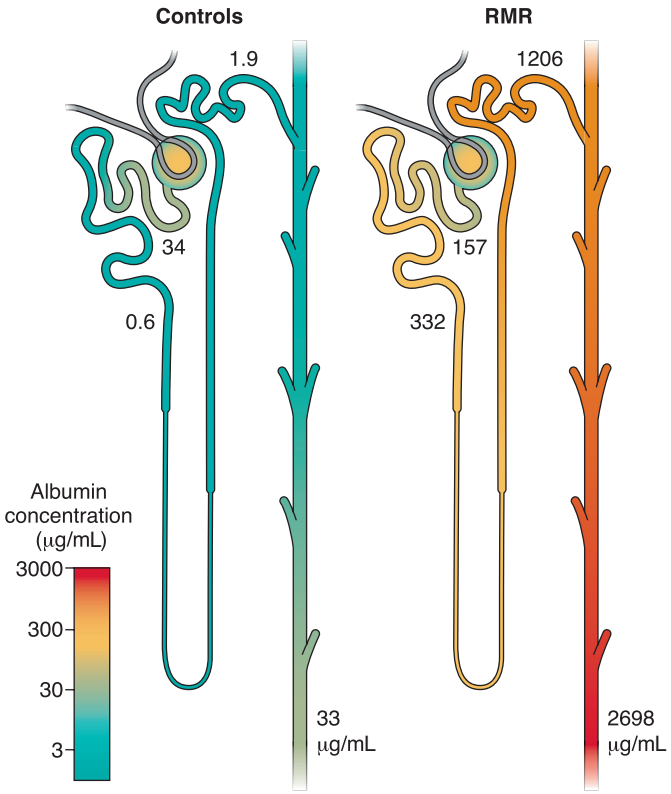


Fig. 30.8 Estimated albumin concentrations along the nephron. Color-coded graphical representation of estimated albumin concentration along the entire nephron in the two animal groups (control group and renal mass reduction group). Numbers represent local group average albumin concentration in μg/mL. *RMR*, Renal mass reduction. (Modified from Sangalli F, Carrara F, Gaspari F, et al. Effect of ACE inhibition on glomerular permselectivity and tubular albumin concentration in the renal ablation model. *Am J Physiol Renal Physiol.* 2011;300: F1291–1300.)

determining its cellular components has been more controversial. The traditional concepts, largely from immunohistochemical studies, indicate that the multilayered cellular lesions are a mixture of glomerular parietal epithelial cells, macrophages, and myofibroblasts,[139-141] the proportion of such cells in the lesion being variable. In both animal models and human tissues, PECs predominate when the Bowman capsule is intact. Recently, a heterogeneous population of renal progenitor cells, previously identified in the normal human Bowman capsule,[142] has been documented in hyperplastic lesions of human crescentic glomerulonephritis.[143] The extracapillary lesions could therefore be the result of dysregulated proliferation of renal progenitor cells in response to the injured podocytes.[143] This possibility is supported by findings in Munich Wistar Frömter (MWF) rats, which are genetically programmed to develop renal damage characterized by excessive progenitor cell migration and proliferation, leading to their accumulation into cellular lesions and glomerulosclerosis.[144] Among the factors that influence the maladaptive PEC response, ultrafiltered albumin has been regarded as a critical player that impairs podocyte regeneration.[145] Notably, albumin prevents PEC differentiation into podocytes by sequestering retinoic acid and impairing retinoic acid response element-mediated transcription of podocyte specific genes.[145]

Circulating components of the complement system are also lost in the urine in proteinuric conditions and become activated at the glomerular level, favoring progression of the lesions.[80,146-148] Thus abnormal fixation of ultrafiltered complement C3 is detected in podocytes showing signs of dedifferentiation and injury during the early stage of proteinuric disease in rats with remnant kidney,[80] and in mice with protein overload proteinuria.[148] C3-deficient mice with protein overload are protected against podocyte structural damage and sclerosis, indicating that C3 might increase susceptibility to injury. Recently, we demonstrated that complement activation via alternative pathway is a pivotal trigger for podocyte loss and PEC activation, leading to glomerulosclerosis in the model of protein-overload proteinuria.[149] Factor H ($Cfh^{-/-}$) or factor B–deficient mice were studied in comparison with wild-type littermates. Wild-type mice with protein overload induced by bovine serum albumin showed podocyte depletion accompanied by glomerular complement C3 and C3a deposits, PEC migration to capillary tuft, proliferation, and glomerulosclerosis. These changes were more prominent in $Cfh^{-/-}$ mice with protein overload induced by bovine serum albumin. The pathogenic role of the alternative pathway was documented by data that factor B deficiency preserved glomerular integrity. In protein-overload mice, PEC dysregulation was associated with upregulation of CXCR4 and GDNF/c-Ret axis. In vitro studies provided additional evidence of a direct action of C3a on proliferation and CXCR4-related migration of PECs. These effects were enhanced by podocyte-derived GDNF. In patients with proteinuric nephropathy, glomerular C3/C3a paralleled PEC activation, CXCR4, and GDNF upregulation. These results indicate that mechanistically uncontrolled alternative pathway complement activation is not dispensable for podocyte-dependent PEC activation resulting in glomerulosclerosis.[149]

Parietal epithelial cells expressing the progenitor cell marker $CD133^{+}CD24^{+}$ have also been reported to proliferate and accumulate into the multilayered cellular lesions in patients with glomerulonephritides characterized by extracapillary proliferation but not in nonproliferative nephropathies such as membranous or diabetic nephropathies.[150] Upregulation of the CXCR4 chemokine receptor on these progenitor cells was accompanied by high expression of its ligand, SDF-1, in podocytes.[150] Moreover, parietal epithelial cell proliferation was associated with increased expression of the Ang II subtype 1 receptor. Renin–angiotensin system blockade normalized CXCR4 and Ang II subtype 1 receptor expression on parietal progenitor cells concomitant with regression of crescentic lesions. Together these findings suggest that the glomerular hyperplastic lesions derive from the proliferation and migration of renal progenitors in response to injured podocytes, and that the Ang II/AT_1 receptor pathway may contribute, together with SDF-1/CXCR4 axis, to the dysregulated response of parietal epithelial cell precursors.

Parietal epithelial cell activation is increasingly recognized and seems to be present also in most forms of FSGS, characterized by nephrotic syndrome often leading to a progressive decline in renal function.[151] Using a lineage tagging approach in mice, it has been documented that activated parietal epithelial cells invade the affected segment of the capillary tuft and initiate glomerular and epithelial basement membrane adhesion and glomerulosclerosis.[152] The Notch signaling pathway has been proposed to play a role in orchestrating parietal epithelial cell phenotypic changes in FSGS.[153] This possibility rests on in vitro evidence that in cultured mouse parietal epithelial cells TGF-β enhanced Notch mRNA expression, which resulted in a significant upregulation of target genes associated with mesenchymal cell phenotype, such as α-smooth muscle actin, vimentin, and Snail.[153] Concurrent inhibition of Notch signaling with the γ-secretase inhibitor DBZ[154] blocked both epithelial-to-mesenchymal transition gene expression changes and cell migration in response to TGF-β, demonstrating a dependence on Notch signaling for induction of mesenchymal gene marker activation in the parietal epithelial cell line. Moreover, in LMB2 antibody-treated NEP25 transgenic mice, a model of collapsing FSGS,[155] Notch inhibition in vivo significantly decreased parietal epithelial cell lesions, pointing to the role of Notch-mediated cell activation in the formation of such lesions.[153]

miRNAs have emerged as important regulators of gene expression in parietal epithelial cells.[96] Indeed, robust miR-150 expression in PECs and podocytes of patients with lupus nephritis correlated positively with disease chronicity scores and the expression of profibrotic proteins. Increased miR-150 would foster the production of profibrotic molecules through the downregulation of its predicted target, SOCS1. The latter protein acts as a negative regulator of the JAK/STAT signaling pathway, which promotes the transcription of genes involved in cell proliferation, inflammation, and fibrosis.[156,157] Similar to miR-150, we identified miR-324-3p increased expression in PECs as well as podocytes in an FSGS rat model. Increased expression of miR-324-3p was associated with the downregulation of its target propyl endopeptidase—involved in the formation of the antifibrotic peptide Ac-SDKD—in fibrotic areas of the kidneys of diseased rats.[158] Human PECs isolated from naïve Bowman capsules express significant levels of miR-193a, which works as a suppressor of podocyte differentiation by inhibiting the expression of the transcription factor Wilms tumor protein (WT1).[159,160] WT1 is essential for the development and maintenance of podocytes and glomeruli by inducing the expression of genes governing podocyte architecture.[161] The downregulation of miR-193a is associated with PEC transdifferentiation toward a podocyte phenotype, whereas its overexpression leads to their abnormal activation, a prerequisite for the formation of crescents in proliferative glomerulonephritis.[160] In line with this, isolated glomeruli from individuals with FSGS are characterized by increased expression of miR-193a, compared with normal kidneys or kidneys affected by other glomerular diseases.[159] The involvement of miR-193a in the pathogenesis of FSGS was also supported by data from miR-193a knock in mice, which develop FSGS with extensive podocyte foot process effacement.[159] All of the previous evidence, together with findings that the number of crescents was reduced by anti-miR-193a in a mouse model of nephrotoxic nephritis,[160] concur to indicate that miR-193a is a promising therapeutic target for FSGS. The mouse model of nephrotoxic nephritis was also instrumental for identifying the key role of miRNAs in orchestrating T-cell–mediated crescentic glomerulonephritis. Mice with miRNA-deficient $CD4^{+}$ T cells develop less severe glomerulonephritis upon toxin injection. Moreover, the kidneys of patients with ANCA-associated crescentic glomerulonephritis and mice with nephrotoxic nephritis are characterized by the upregulation of miR-155 that drives the Th17 immune response and tissue injury.[162]

LOSS OF GLOMERULAR CAPILLARIES: POSTGLOMERULAR HYPOXIA

Irrespective of the underlying process leading to glomerular endothelial damage, such as increased intracapillary pressure and/or podocyte loss, the net result is rarefaction of glomerular capillaries. Loss of glomerular capillary loops translates into diminished postglomerular blood flow from affected glomeruli and downstream injury of the peritubular capillary network. Microvascular dysfunction causes progressive scarring of renal tissue by creating hypoxic environment that triggers a fibrotic response in tubulointerstitial cells.[163] This, in turn, has an impact on adjacent unaffected capillaries and glomeruli, further extending the hypoxic area, and leading to a vicious cycle of progressive destruction of the kidney and decline of renal function to end-stage organ failure.

Indeed, in animal models of proteinuric chronic kidney disease, including anti-Thy1 glomerulonephritis, 5/6 remnant kidney, diabetic nephropathy, and adriamycin-induced nephrosis, the immunohistochemical detection of hypoxia-dependent pimonidazole protein adducts has revealed that renal tissue hypoxia is present early in the course of the disease.[164] Moreover, blood oxygen–dependent magnetic resonance imaging has shown hypoxia in diabetic nephropathy.[165] Although data from animal models provide a compelling argument for postglomerular hypoxia in proteinuric diseases as a primary mediator of progressive renal scarring, data in humans are scarce. Nevertheless, that hypoxia-related injury also applies to humans may be deduced by the finding that there is increased expression of hypoxia-inducible factor (HIF)—a key regulator of the adaptive response to hypoxia controlling expression of hundreds of genes[166]—in biopsies of patients with diabetic nephropathy, IgA nephropathy, and chronic allograft nephropathy.[167]

TUBULAR DAMAGE

Glomerular ultrafiltration of excessive amounts of plasma protein-associated factors incites tubulointerstitial damage and further promotes the effects of glomerular disease on the tubular compartment. The noxious substances in the proteinuric ultrafiltrate may set off tubular epithelial injury with tubular apoptosis, secondary generation of inflammatory mediators, and peritubular inflammation.[4] The mechanisms whereby increased urinary protein concentration leads to nephrotoxic injury are multifactorial and involve complex interaction between numerous pathways of cellular damage (Fig. 30.9).

TUBULAR CELLS: APOPTOSIS AND TUBULO GLOMERULAR DISCONNECTION

Emerging evidence suggests that proteinuria causes tubule cell apoptosis. In cultured proximal tubule cells, delipidated albumin-induced apoptosis in a dose- and time-dependent manner,[168] as characterized by internucleosomal DNA

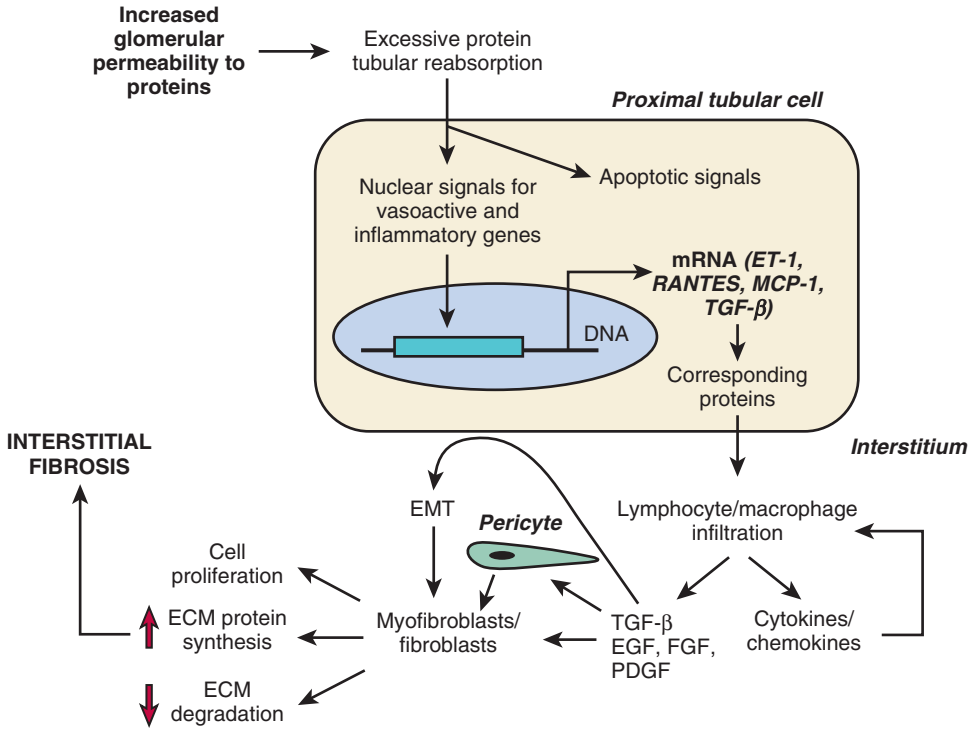


Fig. 30.9 Mechanisms of tubulointerstitial damage induced by proteins. Protein overload of proximal tubule cells as a consequence of increased glomerular permeability to proteins activates intracellular signals that promote cell apoptosis or cause increased production of inflammatory and vasoactive mediators and growth factors. These substances are released into the interstitium, inducing progressive inflammation and injury. *ECM*, Extracellular matrix; *EGF*, epidermal growth factor; *EMT*, epithelial-to-mesenchymal transdifferentiation; *ET-1*, endothelin-1, *FGF*, fibroblast growth factor; *MCP-1*, monocyte chemoattractant protein-1; *PDGF*, platelet-derived growth factor; *RANTES*, regulated upon activation, normal T cell expressed and secreted; *TGF-β*, transforming growth factor-β.

fragmentation, morphologic changes including cell shrinkage and nuclear condensation, and plasma membrane alterations.[168] Kidneys of rats with albumin-overload proteinuria[169] or with passive Heymann nephritis[64] showed increased numbers of terminal dUTP nick-end labeling-positive apoptotic cells in the tubulointerstitial compartment. In tubules, most of the positive cells expressed Ang II subtype 2 (AT2) receptors.[169] Findings of reduced phosphorylation of extracellular signal-regulated kinase (ERK) and Bcl-2 suggested an AT2 receptor–mediated mechanism underlying tubular cell apoptosis.[169]

Apoptotic cells expressing both proximal and distal tubular phenotypes were detected in biopsy specimens from patients with primary FSGS.[170] A strong positive correlation was found between proteinuria and incidence of tubular cell apoptosis.[170]

Renal proximal tubule cells have a remarkable ability to reabsorb large quantities of albumin through clathrin- and megalin receptor–mediated endocytosis.[65] Megalin is the sensor that determines whether cells will be protected from or injured by albumin. It has been shown that megalin binds the serine/threonine kinase PKB, crucial for the phosphorylation of Bad, the Bcl2-associated death promoter.[171] Low concentrations of albumin lead to activated PKB and phosphorylation of the Bad protein, which inhibits apoptosis.[172] On the other hand, overload of albumin leads to decreased megalin expression on the plasma membrane of proximal tubule cells that is associated with reduction of PKB activity and Bad phosphorylation.[173] The result is albumin-induced apoptosis.

In cultured proximal tubule cells, albumin repletion with fatty acids and its association with linoleic acid induced more apoptosis than the exposure to defatted albumin alone.[174] Furthermore, another study showed that nondelipidated albumin or albumin conjugated with palmitate, but not fatty acid-free albumin, altered both tubule mitochondrial variability and membrane potential and caused cytochrome *c* release.[175] In concert with the decline of mitochondrial parameters, fatty acid overload led to a redox imbalance, which deactivated the antioxidant protein peroxiredoxin 2 and caused a peroxide-mediated apoptosis through the redox-sensitive pJNK/caspase-3 pathway. These data were taken to suggest that attempts at lowering circulating fatty acid levels may be important in both preserving redox balance and limiting tubule cell damage.[175] A novel biochemical mechanism has been proposed linking lipotoxicity to tubule apoptosis in proteinuric conditions.[176] The study focused on the Na^+/H^+ exchanger NHE1, a regulator for proximal tubule cell survival through interaction with the membrane phosphoinositide phosphatidylinositol 4,5-bisphosphate (PI[4,5]P2), which initiates formation of a signaling complex that culminates in Akt activation and opposition to apoptotic stress. Starting from the concept that diseased glomeruli with impaired permselectivity allow filtration and proximal tubule reabsorption of nonesterified fatty acids (NEFA) bound to albumin, it was shown that an accumulation of metabolites of NEFA, long-chain acyl-CoA could stimulate lipoapoptosis by competing with the structurally similar PI(4,5)P2 for NHE1 binding, thus interrupting PI(4,5)P2 prosurvival activity.[176]

Proximal tubule cell apoptosis was found to contribute to tubulo glomerular disconnection and atrophy in response to proteinuria in animal models of proteinuric nephropathies.[64] Injured or dying cells release molecules that serve as danger signals.[177] Danger molecules trigger inflammation by engaging pattern recognition receptors such as Toll-like receptors (TLRs)[178] and nucleotide-binding domains, leucine-rich repeat-containing proteins (NLRs),[179] and are thus referred as danger-associated molecular patterns (DAMPs).[180] Through TLRs, DAMPs signal cytokine and chemokine production and upregulate the expression of cell adhesion molecules. When DAMPs interact with NLRs, they stimulate NLRs to complex with apoptosis-associated speck-like proteins to form macromolecular complexes, the inflammasomes that cleave proinflammatory cytokines to their mature forms.[181] Thus besides promoting tubulo glomerular disconnection, proximal tubular cell apoptosis contributes to create a local proinflammatory microenvironment.

TUBULE CELLS: ACTIVATION

Receptor-mediated endocytosis of excessive proteins at the apical pole of the proximal tubule cells is also associated with phenotypic changes characteristic of an activated state.

Insights into specific mechanisms linking protein uptake to cell activation have come from in vitro studies using polarized proximal tubule cells to assess the effect of apical exposure to proteins. Collectively, they show that protein overload induces a proinflammatory phenotype.[182-185] Indeed, upregulation of inflammatory and fibrogenic genes and production of related proteins have been reported following a challenge of proximal tubule cells with plasma proteins. They include cytokines and chemokines, such as monocyte chemoattractant protein-1 (MCP-1), RANTES (regulated upon activation, normal T cell expressed and secreted), interleukin-8 (IL-8), and fractalkine.[182-185] Moreover, levels of the profibrogenic cytokine TGF-β and its type I receptor,[186] tissue inhibitors of metalloproteinase (TIMP)-1 and TIMP-2, as well as membrane surface expression of the αvβ5 integrin[187] were also highly increased in vitro upon stimulation by plasma proteins.

Investigations of the molecular mechanisms underlying chemokine and growth factor upregulation in proximal tubule cells on protein challenge have focused on the activation of transcription factor NF-κB.[182] Other studies confirmed this pathway[188,189] and revealed reactive oxygen as a second messenger.[184,190]

Extrapolation from such in vitro data to the human situation may be difficult considering the conflicting data observed with different proteins in different cell systems,[191] as well as the reported changes in the expression of several genes of unknown function.[192] However, the in vitro observations have recently been confirmed through transcription analysis by cDNA microarray of renal proximal tubule epithelial cells isolated by laser capture microdissection from patients with proteinuric nephropathies.[193] More than 160 genes—including those encoding for signal transduction, transcription, and translation, and apoptotic and inflammatory proteins—were identified as being regulated differently from those in proximal tubule cells from control subjects.

Evidence implicates megalin as a central element of the signaling pathway linking protein reabsorption and gene regulation in proximal tubule cells.[194] Megalin is subjected to regulated intramembrane proteolysis (RIP), an evolutionarily conserved process linking receptor function with transcriptional regulation.[195] Through RIP megalin is subjected to protein kinase C regulated, metalloprotease-mediated ectodomain shedding producing a membrane-associated

C-terminal fragment (MCTF).[194] The MCTF in turn forms the substrate for γ-secretase, which releases the C-terminal, cytosolic domain. The latter translocates to the nucleus where it interacts with other proteins to regulate expression of specific genes. This function may explain the phenotypic change of proximal tubules in proteinuric kidney disease.

That megalin contributes to the early activation of proximal tubule cells in nonselective proteinuria has been documented in megalin knockout/*NEP25* mice given immunotoxin LMB2, a model for nephrotic syndrome, focal segmental glomerulosclerosis, and tubulointerstitial injury.[196] Megalin-deficient proximal tubule cells reabsorbed less proteins in vivo and expressed less tubular injury markers, such as MCP-1 and heme-oxygenase 1.[196]

The proteinuric ultrafiltrate may also activate TLRs and promote an innate inflammatory immune response.[197] Besides being expressed by cells of the immune system such as macrophages, dendritic cells, neutrophils, B cells, and natural killer cells, TLRs are also expressed by nonimmune cells, including renal tubule epithelial cells.[197] The cellular effects of TLRs include the production of proinflammatory cytokines and chemokines that contribute to local inflammation and leukocyte accumulation. It has been demonstrated that proximal tubule epithelial cells were sites of robust expression of TLR-9 mRNA and protein —a receptor for CpG DNA—in NZBxNZW lupus mice with overt nephropathy.[198] Upregulation of TLR-9 expression was accompanied by the development of proteinuria and correlated with tubulointerstitial damage. Furthermore, abundant TLR-9 staining in proximal tubule cells of lupus patients correlated with tubulointerstitial damage. Thus tubular TLR activation may occur due to filtration of plasma proteins, which include immune complexes containing DNA enriched in CG motifs.[199,200]

The proximal tubule bears other receptors for ultrafiltered proteins, such as cytokines and growth factors.[4] Usually these molecules are present in high-molecular-weight precursor forms or bound to specific binding proteins that regulate their biological activity. They can be found in nephrotic tubular fluid. In experimental proteinuria in rats, there is translocation of insulin-like growth factor I (IGF-I) from plasma into tubular fluid (primarily as the 50-kDa complex).[201] Similarly, hepatocyte growth factor (HGF) is present in early proximal tubular fluid from rats with streptozotocin-induced diabetic nephropathy, and it is excreted in the urine of diabetic animals.[202] Under physiologic conditions the high molecular weight of TGF-β complexes prevents glomerular ultrafiltration of this pluripotent cytokine. However, in proteinuric glomerular diseases, TGF-β is present in early proximal tubular fluid and at least a portion is bioactive.[202] IGF-1, HGF and TGF-β are also present in the urine of patients with proteinuric diseases.[203]

Collectively the tubular response to these growth factors can be described as activation or as a moderate change toward a cell phenotype resembling cell injury, which includes a moderate increase in collagen type I and IV production in response to IGF-I[201] and upregulation of the expression of fibronectin by HGF.[204] TGF-β also increases the transcription in proximal tubule cells of the genes encoding collagen α_1III (Col3A1) and collagen α_2 I (Col1A2) as well as fibronectin.

With proteinuria, a putative key factor in tubule cell activation and damage is the excess glomerular filtration of serum-derived complement C3, the central molecule in the complement system that exerts proinflammatory potential.[205] Renal tubule epithelial cells appear most susceptible to luminal attack by the C5b-9 membrane attack complex because of the relative lack of membrane bound complement regulatory proteins, such as membrane cofactor protein (CD46), decay-accelerating factor, or CD55 and CD59 on the apical surface.[206] In rats with severely reduced renal mass[207,208] or with protein overload proteinuria,[148] C3 colocalized with proximal tubule cells engaged in high protein uptake. By limiting the transglomerular passage of proteins, treatment with angiotensin-converting enzyme (ACE) inhibitors was an effective maneuver to reduce C3 load of tubule cells in remnant kidneys.[207] C3 and other complement proteins are also found in proximal tubules in renal biopsy material from nephrotic patients.[205,209] Furthermore, proximal tubule cells are able to synthesize C3 and other complement factors[210] and to upregulate C3 in response to serum proteins in vitro.[211]

The injurious role of plasma-derived C3, as opposed to tubular cell–derived C3, has been documented in C3-deficient kidneys transplanted into wild-type mice.[148] Protein overload led to the development of glomerular injury, accumulation of C3 in proximal tubules, and tubulointerstitial changes. Conversely, when wild-type kidneys were transplanted into C3-deficient mice, protein overload led to a milder disease and abnormal C3 deposition was not observed. Thus ultrafiltered C3 contributes more to tubulointerstitial damage induced by protein overload than locally synthesized C3.

INTERSTITIAL INFLAMMATION AND INJURY

In proteinuric kidney disease, progressive inflammation and injury to the renal interstitium are secondary events following glomerular or vascular injury. Tubule epithelial cells synthesize cytokines and chemokines and accumulate complement components that recruit inflammatory cells and lymphocytes into the interstitium causing progressive fibrosis.

RESIDENT MONOCYTE/DENDRITIC CELLS

The interstitium of normal kidneys contains numerous resident monocytic myelocytes,[212] which express dendritic cell (DC) markers and can indeed present antigens.[212] DCs have recently been described to form an immune sentinel network through the entire kidney, where they probe the environment in search of antigens.[213] An inflammatory environment converts the tolerogenic status of resident DCs into an immunogenic one, favoring recruitment of T cells. It is known that cross-presentation by DCs is a major mechanism for the immune surveillance of tissue against foreign antigens.[214] In this process professional antigen-presenting cells, such as DCs, acquire proteins from other tissue cells through endocytic mechanisms, especially phagocytosis or macropinocytosis. The internalized antigen can then be processed and presented on MHC class I molecules to the extracellular environment.[215] The outcome of cross-presentation regarding immunity depends on the expression of immunostimulatory signals after the uptake of the antigen.[214]

Until recently, the role of resident DCs that accumulate in the renal parenchyma of nonimmune-mediated proteinuric nephropathies remained poorly understood. Recent studies, however, have provided new insights into the activation of DCs in the setting of proteinuria. Administration of ovalbumin—which is freely filtered by the glomerulus—to

normal mice leads to concentration of the protein principally in proximal tubules and to its transfer to DCs in the kidney and renal lymph nodes.[216] Here, ovalbumin is presented to $CD8^+$ T cells, thereby inducing proliferation of these cells.

The importance of kidney DC activation to renal injury has been recently demonstrated by the fact that in transgenic NOH mice (that selectively express the model antigens ovalbumin and hen egg lysozyme in podocytes) DC depletion resolved established periglomerular mononuclear infiltrates.[217] In vitro experiments have also shown that exposure of rat proximal tubule cells to excess autologous albumin, as in the case of proteinuric nephropathies, results in the formation of the N-terminal 24-residue fragment of albumin ($ALB_{1\text{-}24}$).[218] This peptide is taken up by DCs, where it is further processed by proteasomes into antigen peptides. These peptides were shown to have the binding motif for MHC class I and to be capable of activating $CD8^+$ T cells. Moreover, in vivo, in the rat 5/6 nephrectomy model, accumulation of DCs in the renal parenchyma peaked 1 week after surgery and decreased thereafter, concomitant with their appearance in the renal draining lymph nodes. DCs from renal lymph nodes loaded with the albumin peptide $ALB_{1\text{-}24}$ activated syngeneic $CD8^+$ T cells in primary culture.[218] Thus inflammatory stimuli released from damaged tubules after protein overload may represent danger signals that, in the presence of albumin peptides, alert DCs to promote local immunity via $CD8^+$ T cells that are activated in regional lymph nodes and recruited in the renal interstitium.

MACROPHAGES AND LYMPHOCYTES

The interstitial infiltrate of most human chronic renal diseases consists of a number of different effector cells, including macrophages, $CD4^+$ and $CD8^+$ T cells.[219] In animal models, macrophages are the dominant infiltrating cells both in the early and in later stages of chronic renal injury. More specifically, tubulointerstitial macrophage accumulation in chronic nephropathies correlates with the severity of the glomerular and interstitial lesions and the degree of renal dysfunction.[219] Direct damage to resident cells is caused through the generation by macrophages of reactive oxygen species (ROS), nitric oxide (NO), complement factors, and proinflammatory cytokines.[220] Macrophages can also affect the supporting matrix and vasculature through the expression of metalloproteinases and vasoactive peptides.

Macrophages are only one component of the cellular infiltrate that characterizes inflammation in the renal interstitium. Recent models of overload proteinuria have emphasized the importance of tubulointerstitial infiltration with mononuclear cells. Indeed, T-helper cells and cytotoxic T cells, as well as macrophages, are observed in the tubulointerstitial infiltrate 2 weeks after protein overload.[221] T-cell depletion with intraperitoneal anti-T cell monoclonal antibody administration did not modify macrophage infiltration, indicating that the influx of these cells was independent of lymphocytes,[221] and more likely resulted from local tubule cell expression of osteopontin, MCP-1 and the adhesion molecules VCAM and ICAM.

T lymphocytes are also abundantly present in the tubulointerstitial infiltrate early after renal mass ablation in rats and remain there in significant numbers for the following weeks.[222] Although the infiltration of macrophages is part of a nonspecific inflammatory reaction, the presence of lymphocytes within lesions indicates that their recruitment and activation is mediated by an antigen-specific immune response. Their role is to maintain and amplify the inflammatory response in the renal interstitium.

Because B cells are considered to be important mostly in lymph nodes, spleen, and in humoral immune responses, little attention has been paid to their potential role as intrarenal infiltrating cells.[223] However, a prominent accumulation of $CD20^+$ B cells has been described in membranous nephropathy.[224] Furthermore, $CD20^+$ B cells formed a prominent part of the infiltrating cells in renal biopsies from patients with IgA nephropathy and chronic interstitial nephritis.[225] Together with $CD3^+$ T cells, $CD20^+$ B cells formed large nodular structures, like tertiary lymphatic organs in inflamed tissues.[226]

The level of mRNA expression of the chemokine CXCL13 was increased and correlated with $CD20^+$ mRNA in the tubulointerstitial space. The localization of enhanced CXCL13 immunoreactivity to the nodular infiltrates and that of the corresponding receptor CXCR5 to B cells in the infiltrates point toward a role of CXCL13-CXCR5 for B-cell recruitment into the lymphoid follicle-like structures. In the interstitium, B cells may release proinflammatory cytokines and chemokines, present antigens, and activate T cells, as well as play a role in the development of tissue fibrosis.[226]

BONE MARROW–DERIVED FIBROCYTES

In proteinuric renal disease, chemokines generated in the inflammatory milieu may contribute to the recruitment of bone marrow–derived fibrocytes to the renal interstitium.[227] Fibrocytes are circulating connective tissue cell progenitors with a high capacity for collagen I synthesis. In progressive kidney fibrosis induced by unilateral ureteral obstruction in mice, fibrocytes infiltrated the interstitium, and the number of these cells increased with the progression of fibrosis.[228] In addition, the number of infiltrating fibrocytes correlates well with the extent of interstitial fibrosis in several human kidney diseases.[227] Although fibrocytes isolated from mice and humans express chemokine receptors including CCR2, CCR3, CCR5, CCR7, and CXCR4,[227] the specific chemokine and receptor pair involved into the recruitment of these cells in the damaged tubulointerstitium remains uncertain.

FIBROBLASTS: ACTIVATION AND DEPOSITION OF EXTRACELLULAR MATRIX

The process of tubulointerstitial fibrosis involves the loss of renal tubules and the accumulation of myofibroblasts and extracellular matrix (ECM) proteins.[229] Resident interstitial fibroblasts and myofibroblasts proliferate in response to macrophage-derived profibrogenic cytokines, and their number correlates with the subsequent formation of a scar.[230] These cells may be derived from transdifferentiated tubular epithelial cells or pericytes of peritubular capillaries, a process promoted by profibrogenic cytokines, including TGF-β expressed by macrophages.[231,232]

During the developmental stage embryonic epithelia of different organs, including the collecting duct epithelium of the kidney, may give rise to mesenchymal cells, a process known as epithelial to mesenchymal transition (EMT).[233] EMT has been suggested as a process that contributes to interstitial fibrosis in chronic kidney diseases by transformation of injured renal tubule cells into mesenchymal cells.[233]

However, the evidence for EMT in adult kidneys and chronic renal disease is controversial and there are no solid data supporting EMT as an in vivo process in kidney fibrosis. The most supportive data have come from a study in a model of unilateral ureteral obstruction.[234] With the use of genetically tagged proximal tubule epithelial cells, it has been demonstrated that up to 36% of all matrix-producing cells within the tubulointerstitial space may be of tubular origin.[234] However, the contribution of EMT to the formation of myofibroblasts is less in other models.[235,236] Moreover, using cell fate–tracing techniques in the unilateral ureteral obstruction model, other investigators did not find any evidence for a contribution of EMT to renal fibrosis.[237] This is in line with the finding that in the remnant kidney model in rats, after the onset of proteinuria α-smooth muscle actinin, a marker of transdifferentiation to myofibroblasts was initially expressed by nonepithelial cells in the peritubular compartment. Recently peritubular pericytes have been identified as the source of myofibroblasts in this transdifferentiation process.[237]

Activated renal fibroblasts may secrete chemokines which, in turn, may further attract macrophages and perpetuate tubulointerstitial injury.[197] Eventually activated fibroblasts produce interstitial matrix components contributing to interstitial collagen deposition and fibrosis. Increased tubulointerstitial fibrosis is a common feature of kidney injury and results from accumulation of ECM structural proteins. It is maintained by continuous remodeling through the proteolytic action of matrix metalloproteinases (MMPs) and the synthesis of new proteins.

MMPs are inhibited by tissue inhibitors of matrix metalloproteinases (TIMPs). Therefore the balance between TIMPs and MMPs determines the ECM integrity. Among the four members of the TIMP family, TIMP3 is unique in that it is ECM bound and is highly expressed in the kidney.[238] $TIMP3^{-/-}$ mice had more interstitial fibrosis, increased synthesis and deposition of type I collagen, increased activation of fibroblasts, and greater activation of MMP2 after unilateral obstruction than wild-type mice.[239] TIMP3 levels are upregulated in patients with diabetic and chronic allograft nephropathy.[239]

Recent studies link fibrosis to changes in miRNAs.[96,240,241] A number of miRNAs have been shown to be relevant to fibrotic processes in diabetic nephropathy, including miR-29 and miR-200 families, miR-192, and miR-21.[241-244] These miRNAs are regulated by TGF-β in renal cells, and normalization of their expression ameliorated fibrosis in in vitro and in vivo models of diabetes.[243]

More recently, miR-184 has been shown to be a downstream effector of albuminuria driving renal fibrosis in rats with diabetic nephropathy.[245] Indeed, in Zucker diabetic fatty (ZDF) rats, miR-184 showed the strongest differential upregulation compared with lean rats (18-fold). Tubular localization of miR-184 was associated with reduced expression of lipid phosphate phosphatase 3 (LPP3) and collagen accumulation. Transfection of NRK-52E cells with miR-184 mimic reduced LPP3, promoting a profibrotic phenotype. Albumin was a major trigger for miR-184 expression. Interestingly, anti-miR-184 counteracted albumin-induced LPP3 downregulation and overexpression of plasminogen activator inhibitor-1. In ZDF rats, ACE inhibitor treatment limited albuminuria and reduced miR-184, with tubular LPP3 preservation and tubulointerstitial fibrosis amelioration. Albumin-induced miR-184 expression in tubule cells was epigenetically regulated through DNA demethylation and histone lysine acetylation and was accompanied by binding to NF-kB p65 subunit to miR-184 promoter. These results suggest that miR-184 may act as a downstream effector of albuminuria through LPP3 to promote tubulointerstitial fibrosis and offer the rationale to investigate whether targeting miR-184 in association with albuminuria-lowering drugs may be a new strategy to achieve fully antifibrotic effects, at least in diabetic nephropathy.[245]

CHRONIC HYPOXIA

One of the most important contributors to the development of tubulointerstitial fibrosis is chronic ischemia.[246] Production of Ang II and inhibition of production of NO underlie chronic vasoconstriction, which may contribute to tissue ischemia and hypoxia.[247] In that regard, histologic studies on biopsies from animal models and human kidneys have documented that there is often a loss of peritubular capillaries in areas of tubulointerstitial fibrosis.[233] Downregulation of VEGF may be functionally implicated in the progressive attrition of peritubular capillaries and tissue hypoxia, as shown in mouse folic acid nephropathy.[248]

Pericytes play a critical role in the stabilization and proliferation of peritubular capillaries via interaction with endothelial cells.[249-251] This process is mediated by several angioregulatory factors, including angiopoietin-1, produced by pericytes, and angiopoietin-2, produced by activated endothelial cells.[251-253] Renal ischemia, as it occurs in chronic kidney disease due to microvascular rarefaction,[254] promotes an imbalance in angiopoietins that besides leading to proliferation of pericytes may induce interstitial fibrosis in the long term.[255]

Moreover, given that the size of the interstitial compartment determines the diffusion distance between peritubular capillaries and tubule cells, interstitial fibrosis further impairs tubular oxygen supply. Focal reduction of capillary blood flow leading to starvation of tubules may underlie tubular atrophy and loss. Under these conditions, the remaining tubules are subjected to functional hypermetabolism with increased oxygen consumption, which in turn creates an even more severely hypoxic environment in the renal interstitium. In vitro, such hypoxia stimulates fibroblast proliferation and ECM production by tubular epithelial cells.[256]

ENDOGENOUS SYSTEMS OF TISSUE REPAIR

PROTECTIVE MACROPHAGES

Much remains to be learned about macrophages in tubulointerstitial injury. The role of interstitial macrophages was elucidated in mice with progressive adriamycin-induced nephropathy.[257] By treating mice with the monoclonal antibody ED7 directed against the CD11b/CD18 integrin, which is expressed by macrophages, renal cortical macrophages (ED1-positive cells) were reduced by almost 50%, whether ED7 was administered before or after adriamycin administration.[258] However, ED7 reduced renal structural and functional injury only when treatment was started prior to adriamycin administration.[258]

Among several possible explanations for these observations is a temporal change in the predominant macrophage phenotype. If pathogenic macrophages predominated early

and protective macrophages later in the course of the disease, then only early antimacrophage treatment would be expected to protect against progression. Indeed, macrophages can exhibit distinctly different functional phenotypes and can be polarized toward proinflammatory (M1 macrophages) or tissue-reparative (M2 macrophages) phenotype.[259] In the peritubular interstitium, macrophages have been shown to mediate tissue repair in response to acute kidney injury by adopting an M2 phenotype and producing a cytokine environment that supports tubular repair and proliferation rather than inflammation.[260] Colony-stimulating factor-1 (CSF-1) signaling mediated M2 macrophage-induced recovery from renal injury, because pharmacologic blockade of CSF-1 decreased M2 polarization and eventually inhibited tissue repair.[261]

Recent observations also support the importance of macrophage phenotype. For example, in mice with unilateral ureteric obstruction reconstituted with bone marrow of Ang II subtype 1 receptor gene knockout or wild-type mice, infiltrating macrophages were shown to play a beneficial antifibrotic role.[262]

Other studies have demonstrated marked macrophage heterogeneity and context specificity, depending on the nature of the injury and location within the kidney.[263] Evidence is available that macrophages perform both injury-inducing and repair-promoting tasks in different models of inflammation. This has been shown in a reversible model of liver injury, in which the injury and recovery phase are distinct.[264] Macrophage depletion when liver fibrosis was advanced resulted in reduced scarring and fewer myofibroblasts. Macrophage depletion during recovery, by contrast, led to a failure of matrix degradation.[264] These findings provide clear evidence that a functionally distinct subpopulation of macrophages exists in the same tissue.

Further studies on possible temporal variations in the phenotype, activation status, and net effect on injury of macrophages should give a better understanding of the complex role of macrophages in tubulointerstitial injury and repair of chronic renal disease, particularly in the proteinuric setting.

REGULATORY T CELLS

$CD4^+$ T cells constitute a critical component of the adaptive immune system and are typified by their capacity to help both humoral and cell-mediated responses. However, there is a substantial functional diversity among $CD4^+$ T cells, and certain subpopulations hinder rather than help immune response. The most well-characterized example of an inhibitory subpopulation is the $CD4^+CD25^+$, which appears to play an active role in downregulating pathogenic autoimmune responses.[265] $CD4^+CD25^+$ T cells are potent immunoregulatory cells that suppress T-cell proliferation in vitro and have the capacity to suppress immune responses to auto- and alloantigens, tumor antigens, and infectious antigens in vivo.[266]

The regulatory activity of these cells in the setting of chronic renal diseases is highlighted by studies in severe combined immunodeficient (SCID) mice reconstituted with $CD4^+CD25^+$ T cells after induction of adriamycin nephrosis.[258] Mice reconstituted with these regulatory cells had significantly reduced glomerulosclerosis, tubular injury, and interstitial expansion compared with unreconstituted mice with adriamycin-induced nephrosis.

A study using the green fluorescence protein (GFP)-*Foxp3* mouse suggests that *Foxp3* expression identifies the regulatory T-cell population.[267] In the murine model of adriamycin nephropathy, the adoptive transfer of *Foxp3*-transduced T cells protected against renal injury. Urinary protein excretion and serum creatinine were reduced, and there was significantly less glomerulosclerosis, tubular damage, and interstitial infiltrates.[268]

KIDNEY-DERIVED PROGENITOR CELLS

In chronic proteinuric renal disease, regression of glomerular structural changes is associated with remodeling of the glomerular architecture.[269] Instrumental to this discovery were three-dimensional reconstruction studies of the glomerular capillary tuft, which allowed the quantification of sclerosis volume reduction and of capillary regeneration upon treatment.[269] The reversal of early glomerular damage in animal models and humans[270] argues for the existence of a regenerative mechanism that promotes glomerular repair. However, mature podocytes are postmitotic cells with limited capacity to divide in situ and therefore unable to regenerate.[270] A potential mechanism for podocyte replacement by bone marrow–derived stem cells has been described in the Alport mouse model as well as in kidney transplants.[271,272] Nevertheless, most studies concluded that regeneration occurs predominantly from resident renal progenitors,[56,67] although the source of these cells remains ill-defined.

Recently, a study using a triple-transgenic mouse model that allowed permanent marking of glomerular parietal cells and their progeny upon administration of doxycycline showed that parietal epithelial cells of the Bowman capsule possess the capability to migrate into the glomerular tuft via the vascular stalk, where they differentiate into podocytes.[273] Similarly, in the adult human kidney, cells localized between the urinary pole and vascular pole of the Bowman capsule—that expressed both progenitor and podocyte markers ($CD24^+CD133^+PDX^+$cells)—can differentiate into podocytes by losing stem cell markers and expressing markers indicative of a podocyte phenotype while progressing from the urinary pole to the surface of the glomerular tuft.[274]

Experimental evidence indicates that intravenous injection of human progenitor cells harvested from the Bowman capsule into SCID mice with adriamycin-induced nephropathy reduced proteinuria and mitigated chronic glomerular damage.[274] Even more intriguing from the clinical perspective is the finding that ACE inhibition induces glomerular repair in MWF rats, a model of spontaneous glomerular injury.[275] In these proteinuric animals, besides halting age-related podocyte loss, lisinopril increased the number of glomerular podocytes above baseline, which was associated with an increased number of proliferating WT1-positive cells, loss of cycling-dependent kinase inhibitor p27 expression, and increased number of parietal podocytes. This indicates that remodeling of the Bowman capsule epithelial cells contributes to the ACE inhibitor–induced restructuring of the damaged glomerular capillary, primarily by restoring the podocyte population.

Similarly, glomerular repair is augmented when glucocorticoid treatment is given to mice with experimental FSGS at a time when podocyte number was already decreased.[276] Prednisone increased podocyte number, which correlated with reduced proteinuria and decreased glomerulosclerosis. This could be the result of direct biological effects on both

glomerular epithelial cells, by reducing podocyte apoptosis, and by enhancing podocyte regeneration via increasing the number of parietal epithelial cell precursors.

In addition to ACE inhibitors[144,150] and prednisone,[276] Notch inhibitors,[277] blockers of chemokine stromal-derived factor-1[278] and retinoids,[279] can be added to the list of agents that improve podocyte regeneration by augmenting the number of parietal epithelial cells progenitors. Indeed, in vitro exposure of human renal progenitor cells to human serum albumin inhibited their differentiation into podocytes by sequestering retinoic acid and preventing retinoic acid–response element (RARE)-mediated transcription of podocyte-specific genes.[145] Similarly, in mice with adriamycin-induced nephropathy in vivo, a model of human FSGS, blocking endogenous retinoic acid synthesis, increased proteinuria and exacerbated glomerulosclerosis.[145] This effect was related to a reduction in podocyte number. In RARE-lacZ transgenic mice, albuminuria reduced retinoic acid bioavailability and impaired RARE activation in renal progenitors, inhibiting their differentiation into podocytes.[145] Treatment with retinoic acid restored RARE activity and induced the expression of podocyte markers in renal progenitors, decreasing proteinuria and increasing podocyte number, as demonstrated in serial biopsy specimens.[145]

Together these experimental studies suggest that restoring the capacity of parietal epithelial progenitor cells to differentiate into podocytes could promote the regeneration of podocytes and potentially result in the regression of glomerular disease.

SYSTEMIC CONSEQUENCES OF NEPHROTIC-RANGE PROTEINURIA

Nephrotic-range proteinuria is accompanied by a cluster of abnormalities known collectively as the nephrotic syndrome. It is characterized by systemic complications that result from profound alterations in the composition of the body protein pool, a state of sodium retention, dyslipidemia, abnormalities of coagulation factors, and a variable degree of renal insufficiency.

HYPOALBUMINEMIA

Clinical manifestations of the nephrotic syndrome become evident in patients with levels of proteinuria in excess of 3.5 g/day. However, proteinuria in overtly nephrotic subjects usually exceeds this lower bound by a factor of 2 to 3. Immunochemical analysis shows that albumin accounts for more than 80% of the excreted proteins.[280] The second most copiously excreted protein is immunoglobulin, which, after albumin, is the next most abundant protein in plasma. One of the most common systemic abnormalities associated with nephrotic proteinuria is hypoalbuminemia, which develops in most patients.

PATHOGENESIS OF HYPOALBUMINEMIA

Under normal conditions, albumin production by the liver is 12–14 g/day (130–200 mg/kg). Production equals the amount catabolized, predominantly in extrarenal locations.[281] However, about 10%, is catabolized in the proximal tubule of the kidney after reabsorption of filtered albumin.[281] In patients with the nephrotic syndrome, hypoalbuminemia results from excessive urinary loss, decreased hepatic synthesis, and increased rates of albumin catabolism (Fig. 30.10).

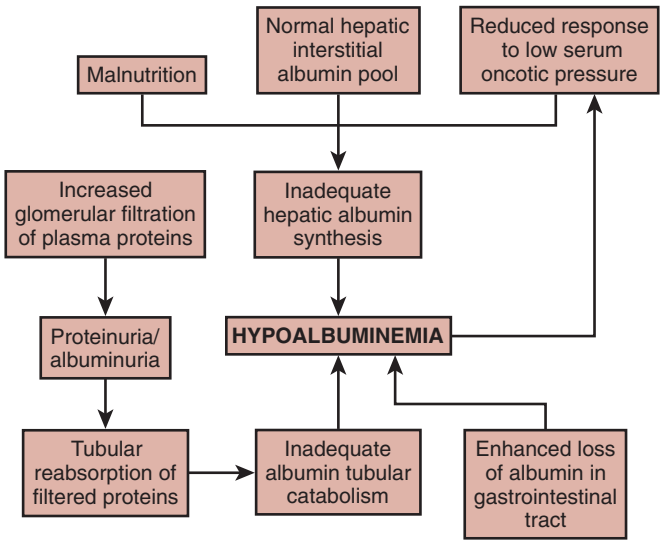


Fig. 30.10 Schematic representation of mechanisms leading to nephrotic hypoalbuminemia. Compensatory mechanisms, such as increase in albumin synthesis and decrease in albumin catabolism, are insufficient to correct the hypoalbuminemia.

Urinary albumin loss is an important contributor to the development of hypoalbuminemia. However, it is not a sufficient cause in most patients with the nephrotic syndrome, as the rate of hepatic albumin synthesis can increase by at least threefold, thereby compensating for urinary albumin loss.[281] Enhanced loss of albumin in the gastrointestinal tract has also been proposed to contribute to hypoalbuminemia, but there is little evidence for this hypothesis.[282] Therefore for hypoalbuminemia to develop there must be either an insufficient increase in hepatic synthetic rate or an increase in albumin catabolism.

Normally the rate of hepatic albumin synthesis may increase by as much as 300%. However, studies of the nephrotic syndrome in animal models and in humans with hypoalbuminemia demonstrate that the rate of albumin synthesis is at or only slightly above the upper limit of normal as long as dietary protein is adequate.[283] This indicates an inadequate synthetic response to hypoalbuminemia by the liver.

Oncotic pressure of the plasma perfusing the liver is one major regulator of protein synthesis.[215] Experimental evidence in rats that are genetically deficient in circulating albumin showed a twofold increase in the hepatic transcription rate of the albumin gene compared with normal rats.[215] However, in these rats the increase in hepatic albumin synthesis was inadequate to compensate for the degree of hypoalbuminemia, which indicates an impaired synthetic response.[215] Similarly, in nephrotic patients, reduced oncotic pressure is unable to enhance the albumin synthetic rate of the liver to the extent required to restore plasma albumin concentration.[283] There is also evidence in normal subjects that the hepatic interstitial albumin regulates albumin synthesis.[284] Because the hepatic interstitial albumin pool is not depleted in the nephrotic syndrome, the albumin synthetic response is normal or slightly increased, but it remains inadequate relative to the level of hypoalbuminemia.[284]

Dietary protein-intake further contributes to the synthesis of albumin. Hepatic albumin mRNA and albumin synthesis was not increased in nephrotic rats when fed a low-protein diet but increased with a high-protein diet.[285] However, serum albumin levels were not altered because hyperfiltration resulting from increased protein intake led to increased albuminuria.

The contribution of renal albumin catabolism to hypoalbuminemia in the nephrotic syndrome is controversial. Some have argued that the renal tubular albumin transport capacity is already saturated at physiologic levels of filtered albumin and that any increase in filtered protein, instead of being absorbed and catabolized, is simply excreted in the urine.[286] Studies in isolated perfused proximal tubules in rabbits, however, demonstrated a dual transport system for albumin uptake.[287] In addition to a low-capacity system that became saturated when the protein load exceeded physiologic levels, a high-capacity low-affinity system was also present and allowed the tubular absorptive rate for albumin to increase as the filtered load rose. Thus an increase in the fractional catabolic rate may occur in the nephrotic syndrome.

This hypothesis is supported by the positive correlation between fractional albumin catabolism and albuminuria in rats with puromycin aminonucleoside-induced nephrosis.[288] Nevertheless, because total body albumin stores are substantially decreased in the nephrotic syndrome, absolute catabolic rates may be normal or even reduced.[282] This outcome is affected by nutritional state, as documented by the fact that in nephrotic rats nourished with a low-protein diet, absolute albumin catabolism was reduced but not in those with normal dietary protein intake.[289]

In summary, hypoalbuminemia in the nephrotic syndrome results from multiple alterations in albumin homeostasis that are not sufficiently compensated for by hepatic albumin synthesis and by decreased renal tubular albumin catabolism.

CONSEQUENCES OF HYPOALBUMINEMIA

Impairment of kidney function is the rule in patients with nephrotic hypoalbuminemia and it usually manifests in two ways. One is the inability of the kidney to maintain sodium and fluid homeostasis. The other is loss of intrinsic ultrafiltration capacity of glomerular capillary walls, a phenomenon that leads, in turn, to fall in GFR.[290]

When viewed in physiologic terms, the GFR can be defined as the net rate of water flux across the walls of the capillaries in the glomerular tufts of the kidney. It is determined by the product of the net pressure for ultrafiltration and the ultrafiltration coefficient—K_f—a measure of intrinsic ultrafiltration capacity, derived from the product of the available filtering surface area(s) and the hydraulic permeability of the glomerular capillary wall (k). By estimating GFR and its determinants in humans, it has been shown that a reduced GFR in some forms of the nephrotic syndrome (minimal change and membranous nephropathy) is exclusively a consequence of profoundly lowered hydraulic permeability.[291] In the nephrotic syndrome associated with lupus nephritis, idiopathic focal and segmental glomerulosclerosis, and diabetic nephropathy, both reduction of the surface area available for filtration and impaired hydraulic permeability contribute to K_f depression.[292, 293]

The principal cause of impaired hydraulic permeability in nephrotic disorders is broadening and effacement of epithelial foot processes.[291] This lowers the frequency of interpodocytic slit diaphragms through which water must pass to gain access to the Bowman space, thereby increasing the resistance to water flow. The low K_f is partially offset by an increase in net ultrafiltration pressure, which is largely due to a substantial lowering of the intraglomerular capillary oncotic pressure. As a result, the fall in GFR is not proportional to the decrease in K_f. This compensatory elevation in net ultrafiltration pressure explains why reduced values of single-nephron GFR are not consistently observed in all experimental nephrotic models.[294]

The low ultrafiltration capacity induced by glomerular disease and protein depletion makes the nephrotic patient particularly vulnerable to acute exacerbations of hypofiltration and renal insufficiency.[295] As the prevailing level of GFR depends heavily on ultrafiltration pressure in the presence of a low K_f, any maneuver lowering the glomerular capillary perfusion pressure can, therefore, cause a precipitous fall in the GFR. The susceptibility of nephrotic patients to episodes of acute kidney injury should thus be borne in mind when prescribing drugs, such as diuretics, cyclooxygenase inhibitors, and cyclosporine, that can compromise the ultrafiltration pressure.

An additional consequence of hypoalbuminemia is the potential for enhanced drug toxicity.[296] Indeed, many drugs are bound to albumin. Hypoalbuminemia reduces the number of available binding sites and increases the proportion of circulating free drugs, but in a steady state this is counterbalanced by faster metabolism. Furthermore, because protein binding may enhance tubule drug secretion, diminished protein binding in the nephrotic syndrome may delay renal excretion of some drugs.[297] Although the clinical consequences of altered protein binding may be difficult to predict, higher levels of free drugs may be toxic, as shown with prednisolone.[298]

The case of diuretics is intriguing. Resistance to loop diuretics, which often occurs in patients with the nephrotic syndrome, may be due to reduced delivery of the diuretic to its site of action, secondary to hypoalbuminemia. Anecdotal reports suggest that the administration of furosemide with small amounts of albumin (6–20 g) can enhance the response to furosemide in nephrotic patients.[299] These observations are not conclusive, because others did not show a difference in the excretion of intravenous furosemide in the urine of nephrotic patients compared with normal controls.[300] On the other hand, excessive amounts of filtered albumin in the tubule may bind furosemide and make it less effective.[301]

In animals, the inhibition of fractional loop Cl^- reabsorption by furosemide is blunted by the presence of albumin in the proximal tubule, and prevention of albumin–furosemide binding with warfarin and sulfisoxazole partially restored the response to the diuretic.[301] However, these findings were not confirmed in nephrotic patients given sulfisoxazole, raising doubt about the importance of excessive albumin-bound furosemide at the active tubular site in resistance to diuretics.[302] Sodium-retaining mechanisms, such as low effective arterial blood volume and activation of neurohumoral factors, may be relatively more important.

Many binding proteins are lost in the urine in the nephrotic syndrome.[303] Consequently, in patients with the nephrotic syndrome, the plasma levels of many ions (iron, copper, and zinc), vitamins (vitamin D metabolites), and hormones

(thyroid and steroid hormones) are low because the level of protein-bound ligands is reduced. Urinary loss of protein-bound ligands can theoretically cause depletion, but there is little convincing clinical evidence for this, with the possible exception of vitamin D.[304] Indeed, one of the proteins lost in the urine of patients with the nephrotic syndrome is cholecalciferol binding globulin (also known as vitamin D binding protein DBP), which is a 59-kDa protein easily filtered by nephrotic glomeruli.[305] Because 25-hydroxycholecalciferol (25-[OH]D_3) circulates as a complex with DBP, there is also an associated urinary loss of 25-(OH)D_3 in the nephrotic syndrome.[306] The oral administration of ^{3}H-labeled cholecalciferol indicated that the serum half-life of 25-(OH)D_3 was reduced and urinary excretion increased in the nephrotic syndrome.[307] Nevertheless, in general, nephrotic patients have normal to decreased plasma levels of 1,25-dihydroxycholecalciferol (1,25-$[OH_2]_2D_3$).[306]

Although the hypocalcemia of the nephrotic syndrome was once attributed solely to the reduction in protein-bound calcium secondary to hypoalbuminemia, a subset of patients has been noted with hypocalcemia out of proportion to the hypoalbuminemia. In these patients ionized serum calcium is decreased.[308] Secondary hyperparathyroidism is seen in some patients, even in the absence of renal failure, as are changes in bone histology consistent with mixed osteomalacia and osteitis fibrosa cystic bone disease.[309] Not all investigators, however, have observed abnormalities in calcium homeostasis in the nephrotic syndrome.[310] Why only a subset of patients is predisposed to alterations in calcium, vitamin D, and parathyroid hormone homeostasis has not been determined, but it has been suggested that factors such as age, duration of disease, renal function, degree of proteinuria, serum albumin concentration, and corticosteroid therapy might be involved.[304]

Finally, hypoalbuminemia may play a role in platelet hyperaggregability.[311] Because albumin normally binds arachidonic acid, thus limiting its conversion to thromboxane A_2 by platelets, hypoalbuminemia might allow increased platelet arachidonate metabolism to take place, and platelet hyperreactivity may result.[311]

EDEMA FORMATION

The clinical manifestation that most frequently brings the nephrotic patient to medical attention is the formation of edema. This represents an increase in the size of the interstitial fluid compartment. The interstitial fluid accumulates most readily in dependent areas where tissue pressure is low. It thus manifests as periorbital edema upon awakening in the morning, and pedal edema at the end of the day. Even when edema is generalized and massive, a condition referred to as anasarca, it remains most marked in the lower extremities. Not infrequently, anasarca is also accompanied by large effusions into the peritoneal, pleural, and pericardial spaces. The mechanisms responsible for extravascular fluid accumulation in nephrotic patients are complex and only partially understood.

REDUCED PLASMA ONCOTIC PRESSURE

Low colloid oncotic pressure as a result of hypoalbuminemia favors the movement of water from the intravascular to the interstitial space. Under normal conditions, edema formation is halted by expansion and proliferation of lymphatics that increase lymphatic flow, and by reduction of interstitial oncotic pressure due to protein-free fluid accumulation. In addition, the increased hydraulic pressure in the interstitium because of fluid accumulation lowers the transcapillary pressure gradient, further reducing the transudation of plasma fluid into the interstitial space. However, there is no clear evidence of alterations in these normal defense mechanisms against edema formation in nephrotic patients.[312] For example, comparable changes in interstitial and plasma colloid oncotic pressure have been documented during relapse and remission phases in patients with nephrotic syndrome.[312] Moreover, the capillary hydraulic conductivity is elevated in nephrotic patients,[313] possibly due to disruption of the intercellular macromolecular complex between endothelial cells, which enhances capillary filtration capacity and may lead to sustained edema formation.[314] These observations suggest that hypoalbuminemia per se may not be the primary determinant of the severity of edema formation and that intrarenal mechanisms may have a prominent contributory role.

ALTERATIONS IN BLOOD VOLUME

According to the traditional view, lowering of the plasma albumin concentration eventually induces renal sodium and fluid retention in the nephrotic syndrome by causing hypovolemia, the so-called underfill mechanism (Fig. 30.11). Indeed, hypovolemia as a consequence of reduced plasma colloid oncotic pressure triggers a cascade of events that signal the kidney to retain the filtered sodium and water.[315] Thus hypovolemia is the afferent stimulus of a complex

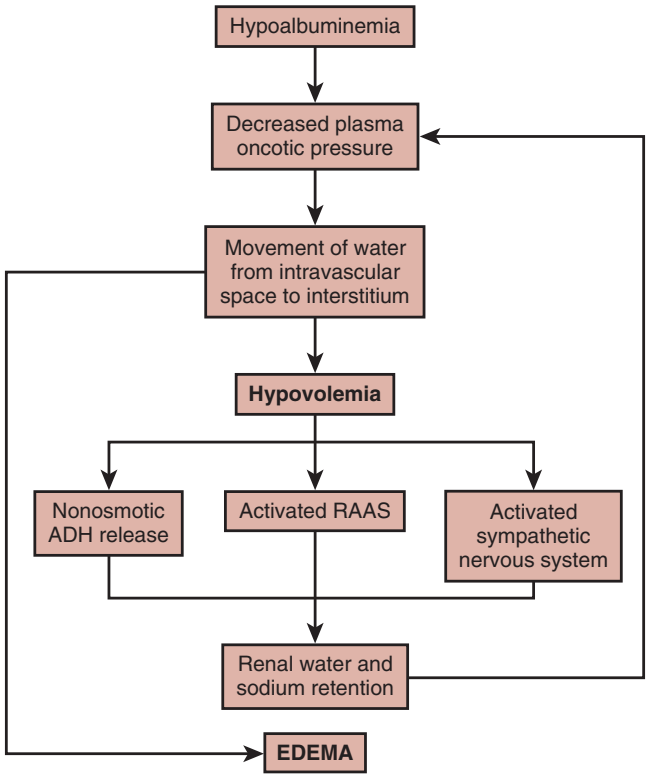


Fig. 30.11 The "underfill" mechanism of edema formation. Hypovolemia, as a consequence of reduced plasma oncotic pressure, is the key event that signals the kidney to retain the filtered sodium and water. *ADH*, Vasopressin; *RAAS*, renin–angiotensin–aldosterone system.

pathway of responses mediated by low- and high-pressure baroreceptors in the cardiac atria, carotid arteries, and aorta that activate the sympathetic nervous system and the renin-angiotensin system. Moreover, hypovolemia also promotes excessive nonosmotic secretion of arginine vasopressin (AVP), which further contributes to water retention by the kidneys.[315]

The homeostatic response of renal sodium and water retention that serves to restore intravascular volume also exacerbates hypoalbuminemia, thereby sustaining transudation of plasma fluid into the interstitial space. The fact that salt retention may be the consequence of an underfilled circulation is consistent with the finding that head-out water immersion, a maneuver that increases plasma volume, is followed by a natriuretic and diuretic response in some nephrotic patients.[316]

This mechanistic scenario of edema formation in the nephrotic syndrome would also imply consistently reduced plasma volume,[317] as well as an elevated plasma renin activity (PRA),[318] and increased plasma and urinary levels of catecholamines.[319] However, only a minority of nephrotic patients have a low plasma volume[319]; in fact, approximately 70% of patients had normal or even high values in some studies.[320] In some cases, plasma volume was lower during remission than during the acute phase of the disease.[319,320] However, methodologic issues have been raised about the measurement of plasma volume in nephrotic patients that may limit the interpretation of these studies.[321]

Measurement of vasoactive hormones, which are responsive to low plasma volume and can be taken as surrogate markers of the intravascular volume, also documented that only 50% of nephrotic patients have higher than normal PRA and plasma as well as urinary aldosterone levels.[322] Moreover, pharmacologic blockade of the renin–angiotensin–aldosterone system in nephrotic patients with a high PRA does not change sodium excretion.[323] Similarly, plasma levels of norepinephrine, arginine vasopressin, and atrial natriuretic peptide (ANP) are near normal or inconsistently changed.[324] The diuretic and natriuretic response to hyperoncotic plasma or albumin infusions,[325] or to central volume expansion with head-out water immersion, also varies widely from patient to patient.[325] Evidence that PRA often increases rather than decreases after steroid-induced remission of the nephrotic syndrome is additional, albeit indirect, evidence that argues against a key role for hypovolemia in edema formation in most nephrotic patients.[322]

INTRARENAL MECHANISMS

Alternatively, the overfill theory hypothesizes that there is a dominant mechanism by which the kidneys retain sodium independently of circulating plasma volume, leading to hypervolemia (Fig. 30.12).[315] Examination of the edema-forming, nephrotic patient during consumption of a known amount of sodium reveals a positive sodium balance. This results in increased blood volume, which by altering Starling forces across the capillary wall, leads to plasma leakage into the interstitium and overflow edema. This mechanism has been illustrated in a unilateral model of puromycin amino–nucleoside (PAN)-induced nephrosis in rats.[326] In such a model, in which albumin concentration in the systemic circulation is normal, only the proteinuric kidney (not the contralateral intact one) retained excessive amounts of sodium and water. This indicates that abnormal sodium retention by the proteinuric kidney is brought about by intrarenal rather than circulating or systemic factors.

These findings can be partly explained by a lowered filtered sodium load, a consequence of the diminished GFR that

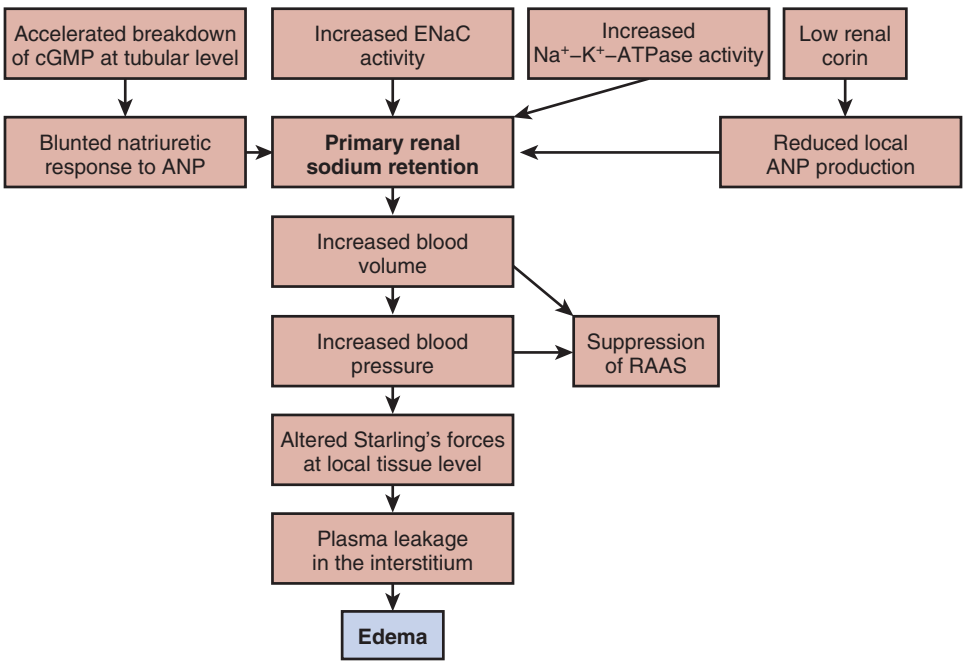


Fig. 30.12 The "overfill" mechanism of edema formation. The abnormal renal sodium retention is the consequence of blunted natriuretic response to atrial natriuretic peptide, increased epithelial sodium channel *(ENaC)* activity, and Na^+-K^+-ATPase activity, and is the key event of the process. The resulting hypervolemia alters Starling forces across the capillary wall at the local tissue level, leading to overflow edema. *cGMP*, Cyclic guanosine monophosphate; *RAAS*, renin-angiotensin aldosterone system.

frequently accompanies nephrotic range proteinuria. However, because the fractional sodium excretion is low, enhanced tubular sodium reabsorption appears to be the predominant cause of sodium retention in the nephrotic syndrome. Analysis of segmental sodium transport in nephrotic rats has identified the collecting duct as the major site of enhanced sodium reabsorption.[326] Refractoriness to the natriuretic action of ANP (which increases urinary sodium and water excretion) in experimental[327] and clinical[328] studies further indicates the distal segments of the nephron as the likely site of sodium retention in nephrotic syndrome. Indeed, the inner medullary segment of the collecting duct is the tubule segment most richly endowed with receptors for ANP.[329]

A crucial observation was that natriuretic and diuretic responses to intravenously infused atrial extract (from normal or nephrotic rats) or synthetic ANP were markedly reduced in nephrotic as compared with normal rats.[330] In a rat model of unilateral glomerulopathy, the blunted natriuretic and diuretic response to ANP was confined to the "nephrotic" kidney as opposed to the contralateral normal kidney, despite a comparable increase in GFR.[327] Moreover, both enhanced release of endogenous ANP during water immersion and infusion of exogenous ANP failed to promote an appropriate natriuretic response in nephrotic patients.[328]

Taken together, these findings support a role for ANP in intrarenal sodium retention in the nephrotic syndrome. In addition, it can be inferred that alterations in the intrinsic transport properties of the collecting duct render this tubule segment unresponsive to the natriuretic action of ANP.

In some studies, increased activity of efferent sympathetic nerve has been related to the blunted ANP natriuretic response.[331] More consistent evidence indicates enhanced phosphodiesterase activity in collecting duct cells from nephrotic animals, leading to accelerated breakdown of normally produced cyclic guanosine monophosphate (cGMP), which is important for intracellular signaling after ANP binding to its specific receptors.[332]

With the discovery of corin, a 1042–amino acid transmembrane serine protease that converts proANP and proBNP into the active forms ANP and BNP,[333,334] the pathogenesis of edema formation in nephrotic syndrome has been revised. In addition to being initially localized in the heart,[333] more recently, corin has been shown to also be expressed in renal tissue.[335] Using immunohistochemical analysis, colocalization of corin and ANP in renal tissue has been documented.[335] It is noteworthy that kidneys of corin$^{-/-}$ mice displayed increased amounts of renal β-epithelial Na^+ channel (ENaC), phosphodiesterase 5 (PDE5), and protein kinase G II, as compared with wild-type mice. Induction of nephrotic syndrome by puromycin aminonucleoside or glomerulonephritis by anti-Thy1 induced concomitant increase in proANP and decrease in ANP in the kidney in association with low renal immunoreactive levels of corin.[335,336] Upregulation of PDE5 and kinase G II resulted in reduced cGMP in the collecting duct, and subsequently in increased ENaC abundance seen in nephrotic syndrome and glomerulonephritis.[336] These findings suggest that corin deficiency by lowering locally produced ANP might be involved in primary salt retention seen in edematous glomerular diseases.[337] In this regard, reduced urinary corin levels were reported in patients with chronic kidney disease.[338]

In the kidney, the ultimate regulation of sodium reabsorption occurs in the collecting duct through the low-conductance epithelial sodium channel (ENaC),[339] located on the apical membrane of principal cells. Evidence is also available that the proteolytic removal of an inhibitory domain from the γ-subunit of ENaC by the serine protease plasmin can activate ENaC.[340] Plasmin, present in the urine of nephrotic rats and humans, has been shown to activate ENaC via this mechanism.[341] Additionally, urokinase-type plasminogen activator present in the rat and human kidney can convert inactive plasminogen (which is filtered by the nephrotic kidney) to the active form plasmin.[341] In the rat PAN nephrosis model, amiloride increased urine sodium excretion and reduced ascites volume. This effect was attributed to the ability of amiloride to inhibit both ENaC and urokinase-type plasminogen activator, and thus to reduce the amount of active plasmin present.[341]

ENaC is also regulated by aldosterone.[339] In rat models of the nephrotic syndrome, activation of ENaC together with elevated plasma aldosterone levels has been reported.[342] Nevertheless, in puromycin-induced nephrosis in rats with clamped aldosterone plasma levels, sodium retention persisted even when ENaC recruitment to the apical membrane was inhibited.[343] Conversely, the transport activity of sodium–potassium–adenosine triphosphatase (Na^+-K^+-ATPase), the ubiquitous sodium pump localized exclusively on the basolateral membrane, was increased.[343] These findings indicate that increased Na^+-K^+-ATPase activity is the driving force behind enhanced sodium reabsorption in the nephrotic syndrome, an observation confirmed by several studies in the cortical collecting ducts in nephrotic rats.[344]

Because the Na^+-K^+-ATPase pump in the basolateral membrane promotes secondary passive sodium entry from the lumen through the ENaC, Na retention in the collecting duct of nephrotic rats can result from the coordinated overactivity of these tubular sodium transporters. A role for the proximal tubule in the avid sodium retention of nephrotic syndrome has been proposed based on the observation that in PAN-nephrotic rats increased sodium reabsorption was associated with a shift of the apical Na^+/H^+ exchanger isoform 3 (NHE-3) from an inactive to an active pool.[345] The increase in NHE-3-specific activity may be a response to the increased albumin load presented to the proximal tubule, as indicated by the correlation between albumin exposure and enhanced NHE-3 abundance and activity in opossum kidney (OKP) cells.[346]

An additional hypothesis concerning intrarenal mechanisms of nephrotic edema proposes that interstitial inflammation of the kidney plays a major role in the pathogenesis of primary sodium retention.[347] The generation of vasoconstrictor and the reduction of vasodilator substances in the interstitium, driven by the inflammatory cell infiltrate, can lead to a reduction in Kf and single-nephron GFR. These glomerular hemodynamic changes that reduce filtered sodium load combine with the increased net tubular sodium reabsorption induced by mediators released from the inflammatory cell infiltrate leading to primary sodium retention, an overfilled intravascular volume, and increased capillary hydrostatic pressure. The decrease in plasma oncotic pressure again promotes fluid movement out of the vascular compartment, thereby buffering the changes in blood volume induced by primary sodium retention. A renal inflammatory infiltrate is, however, minimal or absent

in most children with minimal-change nephrotic syndrome. Thus the nephrotic edema may derive from a combination of primary sodium retention and relative arterial underfilling. The predominance of one or the other mechanism is perhaps in accordance with the pathogenesis of nephrotic syndrome or the stage of the disease.

Deranged renal water handling is also a cardinal feature of the nephrotic syndrome. Defects in both urinary diluting ability[348] and concentrating capacity[348] have been documented in nephrotic patients. The cause of the concentrating defect has been explored in experimental models of the nephrotic syndrome. The extensive downregulation of the expression of the water channels aquaporin 1, 2, and 3 in the collecting duct,[349] and of the urea transporter,[350] as well as a marked decrease in the abundance of thick ascending limb Na^+ transporters represent an appropriate renal response to the extracellular volume expansion observed in the nephrotic syndrome despite increased circulating vasopressin, but may occur at the expense of decreased urinary concentrating capacity.

HYPERLIPIDEMIA

Both quantitative and qualitative changes in lipid metabolism occur in the nephrotic syndrome, with virtually all plasma lipid and lipoprotein fractions being elevated.[351] Blood levels of cholesterol are almost always increased and continue to rise as the severity of the nephrotic syndrome increases.[351] Total cholesterol and cholesterol esters are all increased.[352] Levels of triglycerides are more variable and in many patients do not increase at all, except when the nephrotic state is very severe.[351] Plasma levels of free fatty acids are within normal limits in the nephrotic syndrome, although a smaller than normal fraction is bound to plasma albumin.[351] Levels of very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL) increased early in the nephrotic syndrome[351,353]; data on high-density lipoproteins (HDLs) are less clear. Plasma levels are usually normal but may decrease due to HDL excretion in the urine in severely proteinuric patients.[351]

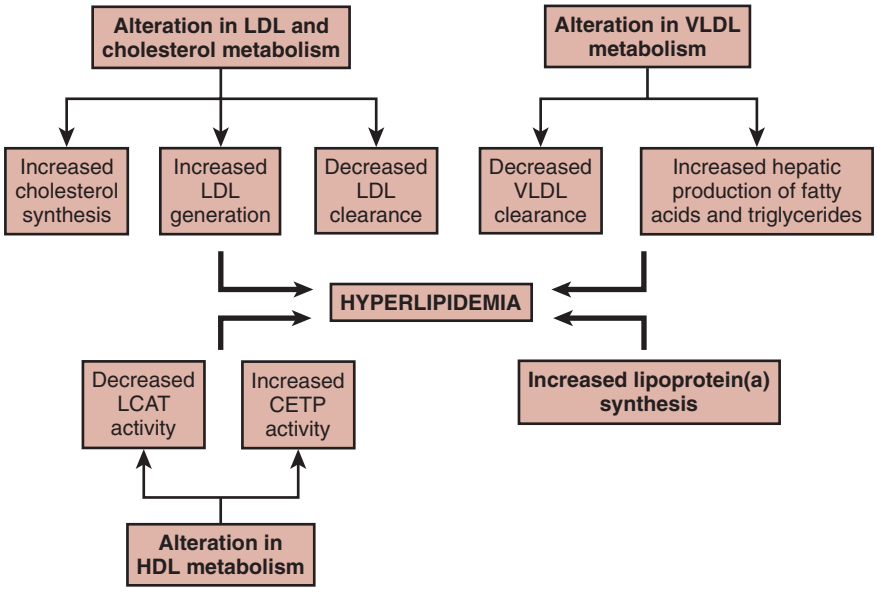
The composition of the lipoprotein molecules is also abnormal. Greater than usual amounts of cholesterol and triglycerides are present in VLDL, IDL, and LDL. Moreover, an alteration in the specific type and quantity of various apoprotein moieties in the lipoprotein molecules has been described, with reduced apo C despite elevations in apo B, C-II, and E and an increased ratio of apo C-III to apo C-II.[354] These abnormalities return to normal promptly when the nephrotic syndrome remits.

PATHOGENESIS OF NEPHROTIC HYPERLIPIDEMIA

Two mechanisms contribute to nephrotic dyslipidemia: overproduction and impaired catabolism/clearance of serum lipids and lipoproteins (Fig. 30.13). There is general agreement that hepatic lipid and apolipoprotein synthesis are both increased and that the clearance of chylomicrons (CM) and VLDL[355] is reduced in the nephrotic syndrome. Cholesterol synthesis has been shown to increase both in animals and humans in response to the hypoalbuminemia associated with the nephrotic syndrome.[356] Hepatic activity of hydroxymethylglutaryl CoA reductase, the rate-limiting step for hepatic synthesis of cholesterol, is elevated.[320] In general, serum cholesterol levels are inversely proportional to serum albumin levels,[320] and cholesterol levels generally normalize upon remission. Conversely, triglyceride synthesis does not appear to be increased.

It has been suggested that lipoprotein synthesis increases in parallel with albumin synthesis because they share a common secretory pathway.[357] This hypothesis was supported by studies showing that infusion of albumin partially corrected nephrotic hyperlipidemia. It has also been reported that apolipoprotein B (apo B) secretion by cultured hepatocytes can be reduced by increasing the oncotic pressure of the culture medium.

Most evidence still indicates that reduced extracellular albumin concentration and/or reduced extracellular oncotic



Fig, 30.13 Pathophysiology of nephrotic hyperlipidemia. All abnormalities of lipid profile originate from alteration in low-density lipoprotein *(LDL)*, very-low-density lipoprotein *(VLDL)*, high-density lipoprotein *(HDL)*, and cholesterol metabolism and increased synthesis of lipoprotein(a).

pressure in some way regulate apolipoprotein synthesis and lipogenesis by the liver. Although hepatic apolipoprotein synthesis is increased in the nephrotic syndrome, not all apolipoproteins are affected to the same degree, and mechanisms causing increased synthesis of the various apolipoproteins are also different. Secretion of apo A is increased approximately sixfold,[357] whereas synthesis of apo B and E is only increased twofold. Synthesis of the apo C is not increased.

Apo A-I mRNA is increased transcriptionally in the livers of nephrotic and analbuminemic rats,[358] suggesting that reduced plasma oncotic pressure or albumin concentration is responsible for the change in apo A-I gene expression. Although plasma apo B and E are both increased in nephrotic and analbuminemic rats, there is little or no change in transcription rates. Thus if increased synthesis is causing increased levels of these apolipoproteins in plasma, the mechanisms involved are most likely posttranscriptional, at the level of translational or protein processing, in contrast to apo A-I.

In addition to increased synthesis, studies in animals and humans have determined alterations in the catabolism of lipids in the nephrotic syndrome. The clearance of chylomicrons and VLDL is reduced following the onset of proteinuria but is normal in rats with hereditary analbuminemia,[357,359] suggesting that urinary loss of a liporegulatory substance, and not reduced albumin concentration or oncotic pressure, may play a role in causing defective lipolysis.

One possible explanation for the defective removal of lipoproteins is a decrease in the activity of lipoprotein lipase (LPL), which hydrolyzes triglycerides in VLDL and chylomicrons, releasing free fatty acids. LPL activity is reduced in nephrotic rats, which provides a potential mechanism for delayed lipolysis.[357] Chylomicron catabolism in hearts isolated from nephrotic rats was decreased in vitro and the LPL pool bound to vascular endothelium was reduced by approximately 90%. LPL activity not bound to the vascular endothelium, and hence unable to interact with large lipoproteins, was normal.[359] Thus a specific reduction in LPL attached to the vascular endothelium may play a role in the reduced catabolism of chylomicrons and VLDL in the nephrotic syndrome.

The relationship between reduced endothelial-bound LPL activity and reduced catabolism of chylomicrons and VLDL is by no means clear. VLDL and chylomicron catabolism by analbuminemic rats is normal despite a marked reduction in heparin-releasable LPL activity.[360] Moreover, it has been reported that HDL isolated from normal animals corrects defective lipolysis of VLDL isolated from nephrotic rats, whereas HDL isolated from nephrotic animals may be dysfunctional. Indeed, HDL isolated from nephrotic animals has been found to be structurally abnormal.[361] Thus multiple separate defects in the peripheral catabolism of triglyceride-rich lipoproteins may be responsible for delayed lipolysis.

Studies in patients with the nephrotic syndrome have not been as detailed as in the rat; however, when comparable studies are evaluated both species exhibit similar disturbances in lipid metabolism. The fractional turnover rate of triglycerides is reduced in nephrotic subjects compared with controls, and the half-life of triglycerides in VLDL is prolonged from 4 to 11 hours.[362] Not only is VLDL catabolism decreased, but the concentration curve over time has an unusual shape, presumably resulting from a delay in the conversion of VLDL into LDL.[363]

It has been suggested that the delay in lipolysis in humans, as in rats, is due to a decrease in LPL activity. Evidence supporting this hypothesis is that LPL activity is reduced in children with the nephrotic syndrome and increases after remission. Furthermore, there is a strong inverse correlation between LPL activity and the concentration of triglycerides in the VLDL fraction,[364] although not all investigators report decreased LPL activity in nephrotic patients.[365]

LDL catabolism has been shown to be either normal or reduced[366] in patients with the nephrotic syndrome and only marginally reduced in nephrotic rats.[367] Reduced receptor-mediated LDL clearance has been reported in some clinical studies,[368] which may account in part for elevations in LDL. A defect in LDL receptor translation or enhanced receptor protein turnover has been hypothesized because normal LDL receptor mRNA was found in nephrotic rats despite marked reduction in LDL receptor protein expression in the liver.[369]

The nephrotic syndrome is also associated with abnormalities in the activity of enzymes required for effective function of HDL. Cholesterol ester transfer protein (CETP) catalyzes the transfer of the cholesterol ester–rich core of HDL_2 to VLDL remnant particles creating LDL, and thereby increasing LDL cholesterol at the expense of HDL cholesterol. CETP is increased in the plasma of nephrotic patients and correlates positively with VLDL cholesterol and negatively with HDL cholesterol.[370]

The enzyme lecithin–cholesterol acyl transferase (LCAT) catalyzes the esterification of cholesterol and its incorporation in HDL particles and promotes the conversion of HDL_3 to HDL_2. The observation that HDL_3 is preserved in plasma from nephrotic patients at the apparent expense of HDL_2 suggests that the LCAT activity is reduced in the nephrotic syndrome.[371] However, increased activity of CETP could also explain this pattern of HDL distribution by rapidly cycling the core of HDL_2 to VLDL remnant particles, thus increasing the flux of cholesterol from the surface of nascent HDL into the core of LDL by increased activity of this enzyme. Furthermore, mature HDL also transports a number of apolipoproteins that serve as cofactors. One of these apolipoproteins, Apo C-II, is an endogenous activator of LPL activity. Apo C-II is normally transported by HDL_2 to nascent VLDL and chylomicrons. Apo C-II may be lost in the urine of nephrotic patients, either as free protein or bound to HDL.[372] Additionally, an inhibitor of Apo C-II, Apo C-III, is increased in the nephrotic syndrome; with the resulting decreased Apo C-II–to–Apo C-III ratio, the activity of LPL is significantly decreased.

CLINICAL CONSEQUENCES OF HYPERLIPIDEMIA

The most important consequence of hyperlipidemia is its potential for inducing cardiovascular disease. The changes that occur in blood lipoprotein composition in the nephrotic syndrome,[353] reduced HDL_2 cholesterol, a relative increase in HDL_3 cholesterol, and the massive increase in total cholesterol, mostly found in the LDL, IDL, and VLDL fractions, are likely to increase the risk of atherosclerotic disease. Nevertheless, the presence of additional risk factors for atherosclerosis in nephrotic patients, notably hypertension, hypercoagulability and chronic renal failure, makes it difficult to determine the individual contribution of hyperlipidemia to the increase in risk.

Given the natural history of atherosclerosis, one would predict that the patient with a protracted form of nephrotic syndrome has the highest risk of dying from premature

cardiovascular disease.[373] Accelerated atherosclerosis has been reported in patients with proteinuria and hyperlipidemia and in some studies has been associated with a strongly increased incidence of cardiovascular disease and stroke.[373] One study reported an 85-fold increase in the incidence of ischemic heart disease in nephrotic patients.[374] In another retrospective analysis of 142 patients with proteinuria greater than 3.5 g/day, the relative risk of myocardial infarction was found to be 5.5 and the risk of cardiac death 2.8 compared with age- and sex-matched controls.[373]

A number of studies have indicated a potential role for hyperlipidemia in the progression of chronic kidney disease. It was proposed that filtered lipoproteins might accumulate in the mesangium and promote sclerosis.[375] In animals, lipogenic diets have been shown to induce focal sclerosis, and the extent of glomerular damage correlates with the serum cholesterol; in the obese Zucker rat, focal sclerosis correlates with hyperlipidemia and can be ameliorated by lipid-lowering drugs.[376] Similarly, free and esterified cholesterol was found in the glomeruli of nephrotic rats, and a close correlation was noted between plasma cholesterol levels and the number of sclerosing glomerular lesions.[377]

Whether hyperlipidemia also plays a role in the progression of chronic kidney disease in the human nephrotic syndrome has yet to be determined. Although there is no specific indication to treat the qualitative abnormalities that characterize the lipid disorder of the nephrotic syndrome, if it is anticipated that the duration of hyperlipidemia will be prolonged, it is wise to initiate therapy. Treatment of nephrotic patients with ACE inhibitors[378] results in a decline in both proteinuria and blood lipid levels even if plasma albumin concentration does not increase. The decline in blood lipid levels includes a decrease in total cholesterol, lipoprotein(a), a decrease in VLDL and LDL cholesterol, and a decrease in the activities of CETP and LCAT.[379]

It is prudent to restrict dietary cholesterol and saturated lipids in patients with the nephrotic syndrome. The long-term effects of dietary supplementation with fish oil (rich in omega-3-polyunsaturated fatty acids) are yet unknown and it cannot be recommended as standard treatment except within the context of a controlled investigative trial. If reduction of proteinuria and dietary fat restriction do not effectively reduce hyperlipidemia, a variety of lipid-lowering drugs, including the 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitors (statins), antioxidants, and fibric acid derivatives, may be useful.

HYPERCOAGULABILITY

Urinary loss of some of the proteins involved in the coagulation cascade and the adaptive increased synthesis of others can induce a hypercoagulable state.[380] Although arterial thrombosis has been reported, it is venous thrombosis that occurs with a particularly high incidence in nephrotic subjects.[381]

PATHOGENESIS OF HYPERCOAGULABILITY

In the nephrotic syndrome, there are widespread alterations in synthesis, turnover, and urinary losses of proteins involved both in coagulation and fibrinolysis. The numerous coagulation abnormalities that occur in the nephrotic syndrome are summarized in Fig. 30.14. Alterations in the concentrations of almost every coagulation factor, including zymogens (factors II, V, VII, IX, X, XI, and XII), cofactors (factors V and VIII), and fibrinogen, can occur.[381] Plasma proteins lost in the urine in patients with the nephrotic syndrome include factors IX, X and XII, which become deficient as there is no sufficient increase in synthetic rates.[381] In contrast, proteins of higher molecular weight, including factors V and VIII, and fibrinogen accumulate because of increased synthesis.[380] Levels of factor VIII typically increase as much as two- to three-fold.[380] However, because factor VIII is also an acute-phase reactant, high factor VIII levels may be an epiphenomenon rather than a causal factor in the development of venous thrombosis.

There is an inverse correlation between serum albumin and fibrinogen levels in the nephrotic syndrome.[382] The elevated plasma levels of fibrinogen likely result from increased hepatic synthesis, as catabolism is normal.[383] Hyperfibrinogenemia may contribute to the procoagulant state by providing more substrate for fibrin formation and by promoting platelet hyperaggregability, increased blood viscosity, and red blood cell aggregation. Increased fibrin deposition, however, may

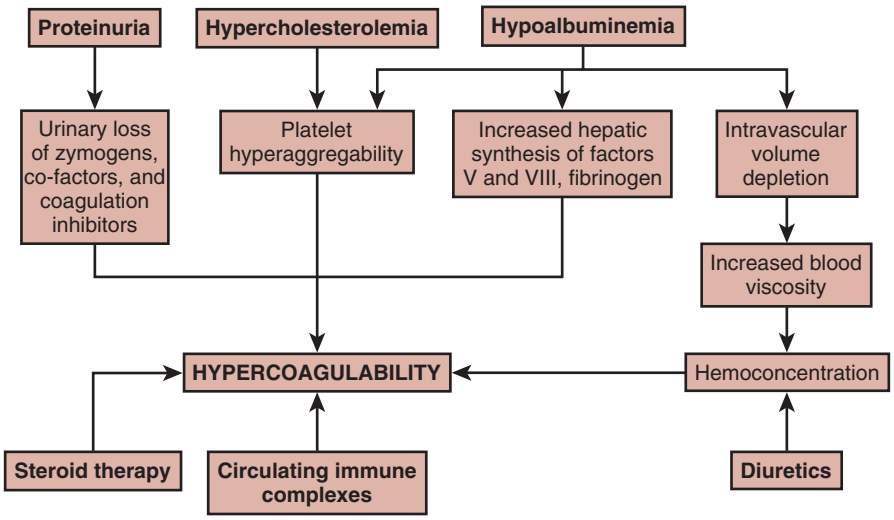


Fig. 30.14 Mechanisms in the pathophysiology of hypercoagulability in nephrotic syndrome. Altered levels and activity of factors in the intrinsic and extrinsic coagulation cascades, levels of antithrombotic and fibrinolytic components of plasma, platelet count and function, and other factors, such as steroids or diuretics, are the numerous abnormalities that contribute to hypercoagulability in the nephrotic syndrome.

also occur due to increased thrombin formation by the elevated levels of factors V and VIII.[384]

Nephrotic patients exhibit abnormalities in endogenous coagulation inhibitors including antithrombin III, which is deficient in 40% to 80% of patients.[385] Plasma levels of antithrombin III correlate negatively with proteinuria and positively with serum albumin levels due to urinary losses of this factor.[385] Antithrombin III deficiency has been associated with serum albumin levels of less than 2.0 g/dL[386] and correlates with deep venous thrombosis and pulmonary embolism in some studies[386] but not in others.[387]

Alterations in other endogenous anticoagulants may also occur in patients with nephrotic syndrome, but the findings are conflicting. Although plasma levels of total protein S are elevated, the active free fraction level is reduced as a consequence of urinary loss, which accounts for the reduction in the activity of this coagulation inhibitor.[388] For protein C results have been contradictory.[388] Levels of tissue factor pathway inhibitor (TFPI) were increased in patients with the nephrotic syndrome in one study despite being of relatively low molecular weight.[389] Two additional factors that may predispose to thrombosis in nephrotic patients are elevations in serum levels of thrombin activable fibrinolysis inhibitor (TAFI), as well as reductions in levels of protein Z.[390]

A number of factors may lead to a reduction in plasmin-induced fibrinolysis in the nephrotic syndrome; much of the work has focused on plasminogen, the precursor for plasmin, and two major regulators of plasmin formation, plasminogen activator inhibitor (PAI -1) and tissue plasminogen activator (t-PA). Several studies noted decreased plasminogen levels in the nephrotic syndrome correlating with the magnitude of proteinuria.[391] Furthermore, hypoalbuminemia itself has been postulated to negatively affect fibrinolysis. Albumin is a cofactor for the binding of plasminogen to fibrin and their interaction with t-PA. One study demonstrated suppressed glomerular fibrinolytic activity in nephrotic syndrome, as there was a sixfold increase in PAI-1, but not t-PA, levels in patients with membranous glomerulopathy compared with controls.[392]

Maintenance of hemostasis also involves the formation of platelet plugs through platelet activation and aggregation. Studies examining platelet abnormalities have suggested a role for enhanced, platelet–vessel wall interaction and platelet aggregation in the development of thromboembolism in nephrotic syndrome. Thrombocytosis, decreased red blood cell deformability, and increased von Willebrand factor levels all favor platelet transport toward the vessel wall and increased platelet adhesion[393] and are observed in the nephrotic syndrome.

In vitro studies have demonstrated increased platelet aggregation in nephrotic patients.[393] In addition to platelet hyperaggregability, markers of platelet activation, including plasma P-selectin levels and circulating CD62P-positive platelets, were higher in nephrotic patients than in healthy controls. Increased CD62P expression was found in pediatric patients during the nephrotic but not during remission.[394]

Platelet hyperaggregability is associated with hypoalbuminemia, hypercholesterolemia, and hyperfibrinogenemia.[394] Hypoalbuminemia results in increased availability of normally albumin-bound arachidonic acid, leading to increased formation of thromboxane A_2 in platelets, which promotes platelet aggregation.[395] Elevated levels of LDL cholesterol may increase platelet aggregation as suggested by the observation that lipid-lowering therapy reverses the spontaneous platelet hyperaggregability seen in such patients.[396] Such an effect, however, has not been conclusively shown in the general population.[397]

To date, observations suggest that platelet activation and aggregation may play a role in the increased risk of thromboembolism in patients with nephrotic syndrome. However, attempts at correlating in vitro functional tests with clinically overt thromboembolic events have shown conflicting results.[387] Other clinical features of the nephrotic state, such as intravascular volume depletion and exposure to steroids, also contribute to hypercoagulability. Increased blood viscosity is associated with hemoconcentration and enhanced using diuretics[398] and by hyperfibrinogenemia.[380] The nature of the underlying immunologic injury may play a role and account for the predilection of thrombosis for the renal vein and for the increased incidence of thrombotic complications in membranous glomerulopathy. The identification of circulating immune complexes in patients with membranous glomerulopathy with renal vein thrombosis, but not in those without thrombosis, supports this possibility.[381] The use of steroids has also been suggested to predispose patients to thromboembolic complications,[381] but other studies have reported a high incidence of thromboembolic complications in the absence of steroid therapy as well.[399]

Thus abnormalities in any of the steps that promote coagulation, including activation and termination of the coagulation cascade, fibrinolysis, and platelet activation and aggregation, may contribute to the hypercoagulable state seen in the nephrotic syndrome. The specific role of each of these alterations remains ill-defined.

CLINICAL CONSEQUENCE OF HYPERCOAGULABILITY

Thromboembolic events are serious complications of the nephrotic syndrome. The most frequent site of thrombosis is the renal vein. Retrospective and prospective studies have shown an incidence of renal vein thrombosis (RVT) in the nephrotic syndrome ranging from 5% to 62%.[381] Nephrotic syndrome is associated with RVT regardless of the underlying disease. Observational studies evaluated patients who underwent renal venography.[400,401] These studies show that the prevalence of RVT is highest in patients with membranous nephropathy, on average 37%. However, the risk is still clinically important in other primary glomerular diseases, particularly in membranoproliferative glomerulonephritis and minimal change disease. Furthermore, the risk of developing RVT may have been underestimated in these largely cross-sectional studies because patients who were initially not found to have RVT may have subsequently developed it during the disease. In the largest prospective study assessing RVT in 151 patients with nephrotic syndrome, the cumulative incidence of RVT was 22%.[400]

RVT presents clinically in two ways,[400] acute and chronic. Acute RVT is usually unilateral and characterized by acute flank pain, flank tenderness, macroscopic hematuria, and some deterioration of renal function. Chronic RVT is usually asymptomatic and occurs in the elderly. Selective renal venography is the gold standard for the diagnosis of RVT, and demonstration of venous collaterals establishes chronicity. However, renal venography is invasive and associated with complications that include pulmonary embolism due to clot dislodgment, inferior vena cava perforation, and contrast-induced acute kidney injury.[402] Consequently, noninvasive

diagnostic tests are preferred, including intravenous pyelography, computed tomography, and magnetic resonance imaging.[402] Nevertheless, there is need for further studies of the usefulness of these techniques in the diagnosis or exclusion of acute RVT remains unproven. Doppler ultrasonography appears to be inferior to renal venography in establishing the diagnosis of RVT and cannot be recommended based on the current data.[403]

Early data on the prognosis of nephrotic patients with RVT suggested a dismal outcome. More recently, it became clear that in the presence of anticoagulation therapy, symptomless chronic RVT is benign.[400] Deep venous thrombosis of the lower extremities is also observed in the nephrotic syndrome and can occur in isolation in up to 15% of patients,[401] or in association with RVT.[400]

Pulmonary embolism may complicate deep vein thrombosis involving the lower extremities, inferior vena cava, or RVT. In a case series of 151 patients with the nephrotic syndrome,[400] of whom 94 patients underwent ventilation–perfusion lung scanning, symptomatic pulmonary embolism was observed in 25% of patients with acute RVT and 20% of patients with chronic RVT. The incidence of asymptomatic pulmonary embolism was 12.8%. In both prospective and retrospective studies, the incidence of thromboembolic complications other than RVT ranges from 8% to 44%, with an average incidence of 20%.[381]

Many clinical studies have demonstrated an association between hypoalbuminemia and venous thromboembolism, but serum albumin levels in patients with and without thromboembolic events were not significantly different.[400] These data suggest that hypoalbuminemia is associated with, but not a prerequisite for, the development of thromboembolic complications in nephrotic patients.

The pattern of thrombosis is remarkably different between children and adults. Despite a lower incidence (1.8%–5%),[404] thromboembolic complications in children tend to be more severe, and half of the children have arterial thrombosis that may cause clinical problems, such as persistent hemiplegia, mesenteric infarction, and peripheral occlusion, leading to amputation.[404] In adults, arterial thrombosis is much less common than venous thrombosis, but it is a serious complication causing important morbidity. One case series described 43 patients with the nephrotic syndrome who had arterial thromboembolism at aortic, renal, femoral, mesenteric, cerebral, or brachial sites.[399] An increased risk of coronary events in patients with the nephrotic syndrome has been documented in a retrospective study.[373]

The treatment of venous thromboembolism in nephrotic patients is similar to that in the general population. First-line treatment consists of conventional anticoagulation with low-molecular-weight heparin and oral vitamin K antagonists.[381] When therapy is initiated early in the course of acute RVT, renal function and other symptoms of RVT have been shown to improve significantly.[400] Oral vitamin K antagonists are usually continued for the duration of nephrotic-range proteinuria,[405] as RVT can recur in the setting of ongoing nephrosis after withdrawal of anticoagulation therapy. Low-molecular-weight heparins have a reasonable safety profile but should be used cautiously in patients with renal insufficiency because of excessive anticoagulant activity and an increased risk of bleeding due to drug accumulation.[406]

Controversy exists regarding the use of prophylactic anticoagulation therapy in patients with nephrotic syndrome who do not have RVT. The potential benefit of prophylactic anticoagulant therapy for patients with membranous glomerulopathy has been documented using decision-analysis methodology.[382] Uncontrolled series show a high mortality from pulmonary embolism among patients not receiving anticoagulant therapy and very low rates of RVT and pulmonary embolism in patients given anticoagulant therapy. Prophylactic anticoagulation is warranted as long as the patient has nephrotic proteinuria, an albumin level below 2 g/dL, or both. In patients with other underlying diseases, a more cautious approach may be indicated, and prophylactic anticoagulant therapy should be initiated only if the risk of thromboembolic events is considered high.

SUSCEPTABILITY TO INFECTION

Loss of high filtered protein load through both urinary excretion and tubular catabolism,[407] as well as a reduced rate of synthesis,[408] may result in concurrent deficiency of IgG and components of the alternate complement pathway, including factor B. Indeed, patients with nephrotic syndrome have low serum levels of various IgG subclasses. Also, IgA levels are decreased in nephrotic syndrome, whereas IgM usually is increased, particularly in patients with minimal-change disease and normal renal function.[409] Furthermore, defective cell-mediated immunity has been reported in nephrotic syndrome,[410,411] including reduced number of total circulating T lymphocytes and blunted blastogenic response by lymphocytes to the mitogens concanavalin A and phytohemagglutinin.

The defects in the humoral and cellular-mediated immunity render the nephrotic patient highly susceptible to infection.[412] The organisms most frequently encountered are *Streptococcus pneumoniae* and *Escherichia coli.* Although such susceptibility to infection is generalized, there seems to be a particular vulnerability to local infection at the sites of edema formation. Splits in the skin caused by edema and malnutrition may predispose nephrotic patients to cellulitis.[320] Peritonitis has been reported in patients who have ascitis.[412] It also occurred in approximately 6% of children with nephrotic syndrome who suffered one or more episodes of the infection.[412]

The unusual susceptibility of children to infections with encapsulated microorganisms is associated with urinary losses of the alternate pathway complement components, particularly factor B, or C3 proactivator, and D, which are essential for the destruction of encapsulated bacteria in the absence of specific antibodies.[407] The capacity to opsonize the encapsulated bacteria can be restored to normal by adding pure factor B to nephrotic serum.[407] Fungemia due to *Candida lusitaniae* has been also reported in low–birth-weight premature infants with congenital nephrotic syndrome.[413]

New potent antibiotics have contributed to considerably decreasing the incidence of fatal infections in nephrotic syndrome. However, prophylactic measures, such as pneumococcal vaccine, are recommended in adults with severely depressed immunoglobulin levels and nephrotic children over 2 years of age, especially when early remission of nephrotic syndrome is not anticipated.[414]

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Complete reference list available at ExpertConsult.com.

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BOARD REVIEW QUESTIONS

1. Charge-selectivity in the glomerular basement membrane (GBM) functions in which of the following manners?
   a. Positive charges on the GBM repel positively charged circulating proteins
   b. Positive charges on the GBM attract negatively charged circulating proteins
   c. Negative charges on the GBM attract positively charged circulating proteins
   d. Negative charges on the GBM repel negatively charged circulating proteins

   Answer: d

   Rationale: The majority of circulating proteins including albumin are negatively charged. Evidence from theoretical models and experiments in animals suggests that negatively charged molecules in the glomerular capillary wall (in the glycocalyx of endothelial cells, the negatively charged heparansulfate of the glomerular basement membrane, and the glycoproteins of the cell membrane of podocytes) form an important component of glomerular permselective function.

2. The components of the glomerular capillary membrane wall are
   a. Endothelial cells and GBM
   b. Endothelial cells and basement membrane and podocyte foot process
   c. Endothelial cells and podocyte foot process
   d. Endothelial cells and basement membrane and parietal cells
   e. Endothelial cells and parietal cells and podocyte foot processes

   Answer: b

   Rationale: The glomerular capillary membrane is a complex structure composed of the three layers stated in option b that allow a large amount of water and small solute filtration while efficiently restricting glomerular passage of protein macromolecules within blood circulation.

3. Which of the following molecules are thought to be important in the absorption of filtered proteins by proximal tubule cells? (Select all that apply.)
   a. Uromodulin
   b. Megalin
   c. C3 complement
   d. Clathrin
   e. Nephrin

   Answer: b and d

   Rationale: Renal proximal tubule cells have a remarkable ability to reabsorb large quantities of albumin through clathrin- and megalin receptor–mediated endocytosis. Uromodulin (Tamm–Horsfall protein) is secreted by tubule cells and is thought to play a role in preventing urinary infections. C3 is a component of the complement system that is thought to play an important role in the progressive renal injury resulting from proteinuria. Nephrin is a key component of the glomerular slit diaphragm.

4. Locally produced angiotensin II contributes to glomerular injury by
   a. Sustaining glomerular hypertension–induced damage
   b. Impairing the glomerular barrier sieving function through inhibition of nephrin expression by podocytes
   c. Activating parietal epithelial progenitor cells in the setting of glomerular hyperplastic lesions
   d. All the above mechanisms

   Answer: d

   Rationale: Angiotensin II has been identified as a key mediator of multiple mechanisms that contribute to proteinuria and progressive kidney damage including all those listed.

5. The main systemic consequences of nephrotic-range proteinuria include
   a. Alteration in the composition of the body protein pool—hypoalbuminemia
   b. A state of sodium retention
   c. Dyslipidemia
   d. Abnormalities of coagulation factors
   e. Susceptibility to infection
   f. All the above abnormalities

   Answer: f

   Rationale: The nephrotic syndrome is a complex pathophysiologic state characterized by sodium and water retention as well as multiple abnormalities resulting from excessive urinary loss of albumin as well as other proteins that are components of the coagulation and humoral immune systems.