CHAPTER

76 Anemia in Chronic Kidney Disease

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INTRODUCTION

Anemia in patients with chronic kidney disease (CKD), including end-stage renal disease (ESRD) and kidney transplantation, is a complex condition with important prognostic and therapeutic implications. Anemia is defined by the World Health Organization (WHO) as hemoglobin (Hb) < 13 g per deciliter in men and < 12 g per deciliter in women, and the National Kidney Foundation (NKF) recommends an evaluation when Hb is < 13.5 g per deciliter in men and < 12 g per deciliter in women.[1] Nonetheless, a more precise characterization of anemia in CKD is problematic, and involves consideration of numerous hematologic, gastrointestinal, and hormonal abnormalities. In this chapter, we review the most recent evidence concerning the epidemiology, pathophysiology, diagnosis, and treatment of anemia in kidney disease. When applicable, we distinguish among CKD, ESRD, and transplant populations.

EPIDEMIOLOGY

The prevalence of stages 1 through 4 CKD in the United States has been estimated at 13.1%, or about 40 million people.[2] Anemia is common in CKD, and its prevalence increases as kidney function decreases. Claims data from the 2010 United States Renal Data System (USRDS) include a diagnosis of anemia in 43% of patients with stages 1 to 2 and 57% of those with stages 3 to 5 CKD.[3] African Americans have correspondingly higher rates of 48% and 64% for stages 1 to 2 and 3 to 5 CKD, respectively. In contrast, anemia is present in 12% to 14% of subjects at risk for kidney disease (Kidney Early Evaluation Program [KEEP]) and only 5% to 6% of the general population (National Health and Nutrition Examination Survey [NHANES] 1999 to 2004).[4]

As of December 31, 2008, the combined hemodialysis (HD) and peritoneal dialysis (PD) population in the United States was approximately 381,000 persons.[3] Because the use of erythropoiesis-stimulating agents (ESAs) has decreased (see the following), the mean Hb of incident ESRD patients has fallen from approximately 10.5 g per deciliter in 2006 to 9.9 g per deciliter in 2008. The Kidney Disease Outcomes Quality Initiative (KDOQI) target Hb of 11 to 12 g per deciliter, established in 2007, was achieved by only 37% to 38% of prevalent dialysis patients in 2008.[3] Even considering 6-month rolling averages, this target was consistently achieved by < 50% of HD patients.[5]

Hb variability, defined as the spontaneous change in Hb concentration above or below the desired range with time, is common in CKD and ESRD patients.[6] In the HD population, the standard deviation (SD) of Hb levels around the mean is 1.1 to 1.3 g per deciliter, and only 8% to 18% of patients maintain stable Hb over 6 to 12 months.[7] In contrast, in the general population, the SD of Hb levels is < 0.6 g per deciliter. This variability may[8–10] or may not[11] have important implications for morbidity and mortality in CKD and ESRD patients (see the following). Hb also varies normally with age, sex, and race, as well as under the influence of infection, inflammation, other comorbidities, and HD parameters such as adequacy and water quality.[6,12]

In patients with CKD, anemia is associated with increased all-cause and cardiovascular mortality and higher rates of left ventricular hypertrophy (LVH), congestive heart failure (CHF), major adverse cardiovascular events (MACE), and progression to ESRD.[13] In a cohort of > 1300 men without clinical history of CHF, the presence of both Hb < 13 g per deciliter and glomerular filtration rate (GFR) < 60 mL/min/1.73 m^2, compared to either anemia or CKD alone, was associated with a significantly higher rate of previously unrecognized LV dysfunction (ejection fraction [EF] < 40%).[14] Rates of CHF hospitalization and death were 3 to 5 times higher in the group with anemia and CKD compared to the group with neither anemia nor CKD. Among European HD patients (Dialysis Outcomes and Practice Patterns Study [DOPPS]), the relative risk (RR) for hospitalization and death increased by 4% and 5%, respectively, with each 1 g per deciliter decrease in Hb within the 10 to 13 g per deciliter range.[15] Consistent with these data, in a recent study of HD patients from the United Kingdom Renal Registry, the relative hazard for death increased by nearly threefold with each 1 g per deciliter decrease in Hb within the 9 to 13 g per deciliter range.[16] Among U.S. PD patients, the hazard ratio

(HR) for hospitalization and death increased similarly with decreased Hb within the same range.[17]

Anemia is increasingly recognized as a major cause of signs and symptoms previously attributed to uremia, including fatigue, weakness, dizziness, cold intolerance, shortness of breath, decreased exercise tolerance, and decreased muscle strength.[6,18,19] Anemia in CKD is also associated with functional and mobility impairment, increased risk of falls, and decreased health-related quality of life (QOL).

PATHOPHYSIOLOGY

Anemia: Historical Overview

The Bible and ancient medicine portrayed blood as a symbol of life, and most physical and mental disease was attributed to disorders of the blood.[20] The first xenogeneic (animal-to-human) blood transfusion was reported in Paris in 1667. Physician-in-Ordinary to Louis XIV, Jean-Baptiste Denis, and surgeon Paul Emmerez transfused whole lamb blood into a patient who had previously been bled 20 times for fever, whose symptoms of anemia improved markedly following the procedure. The first successful allogeneic (human-to-human) blood transfusion was reported in London in 1825, when James Blundell transfused a man's blood into his wife to treat postpartum hemorrhage.[20]

The idea that the bone marrow is the site of erythropoiesis in response to hypoxia was first proposed in 1823 and gradually expanded during the following century.[20–22] In 1863, Jourdanet observed that blood viscosity was higher in Mexicans living at high altitudes than in those living at sea level.[23] In 1891, Viault extended this observation to red blood cell (RBC) counts in Peruvians.[24] Carnot and DeFlandre[25] reported in 1906 that serum from bled rabbits, when injected into normal rabbits, stimulated RBC production. They tentatively coined the term "hemopoietine" to identify the presumed erythropoietic substance transferred by the serum. Similar experiments, in which serum or plasma from anemic or hypoxemic rabbits caused a reticulocytosis in recipient rabbits, were conducted from 1932 to 1953.[22] Brown and Roth[26] determined in 1922 that the anemia of chronic nephritis resulted from hypoactive bone marrow. In 1950, Reissmann[27] used parabiotic rats to show that, although only one animal was exposed to low O_2 tension, both partners exhibited bone marrow erythroid hyperplasia, reticulocytosis, and polycythemia, again strongly suggesting the presence of a humoral erythropoietic factor acting on the bone marrow. In 1948, Bonsdorff and Jalavisto[27a] named this factor erythropoietin (EPO).

In subsequent years, the tissue origin of EPO was elucidated. In 1957, it was shown that a bilateral nephrectomy prevented the rise in plasma EPO levels in bled rats.[28] Experiments from 1961 to 1974 showed that perfusates from hypoxic rabbit and dog kidneys could stimulate presynthesized EPO release and subsequent reticulocytosis.[21,22] In 1983, EPO mRNA from hypoxic rat kidneys, injected into frog oocytes, was translated into EPO, and experiments from 1988 to 1996 demonstrated EPO transcripts and biologically active EPO in the peritubular interstitial cells of kidneys from a number of species.[29]

More recent discoveries have included the description of EPO physiology and molecular biology, especially pertaining to kidney disease. Erythropoietic activity was quantified from 1955 to 1961 by the measurement of increased ^{59}Fe uptake by RBCs in polycythemic rats and in normal rats that had been injected with plasma from anemic rats.[30,31] The inverse relationship between EPO concentration and hematocrit was reported in 1968.[32] EPO itself was ultimately purified in 1977 by concentrating 2550 L of urine from patients with aplastic anemia.[33] Isolation of the purified protein facilitated the development of an EPO radioimmunoassay in 1979,[34] followed by the cloning and expression of the EPO gene in 1985.[34,35] Clinical trials of recombinant human erythropoietin (rHuEPO), which showed improved Hb in ESRD patients, were conducted from 1986 to 1987, thereby ushering in the era of ESA therapy for the anemia of CKD.[36,37]

Normal Erythropoiesis

Tissue hypoxia, primarily in the kidney, provides the major physiologic stimulus for RBC production. Importantly, a fall in renal blood flow (RBF) is coupled with a decrease in GFR, which leads to reduced oxygen use by tubule transport proteins. Thus, tissue oxygenation is relatively independent of RBF across a wide range.[38,39]

Hypoxia-inducible factors (HIFs) are helix-loop-helix heterodimeric transcription factors that consist of O_2-regulated α subunits and constitutively expressed β subunits.[39] In normoxic cells, O_2, Fe^{2+}, and ascorbic acid activate prolyl-4-hydroxylase domain (PHD) proteins, which catalyze the hydroxylation of key proline residues in HIF-α subunits.[40,41] HIF-α then binds the von Hippel-Lindau protein, is polyubiquitinated by E3 ubiquitin ligase, and then is swiftly degraded by the proteasome.[20,42] Conversely, in hypoxic cells, low O_2 tension and high concentrations of tricarboxylic acid cycle intermediates inhibit PHD proteins, allowing HIF-α to translocate to the nucleus, heterodimerize, and activate hypoxia response elements (HREs) that regulate gene transcription for numerous cellular processes, including erythropoiesis, angiogenesis, and anaerobic metabolism (Fig. 76.1).

Of the three HIF proteins currently known, HIF-2 is the most strongly implicated in the hypoxic induction of EPO.[39,42] HIF-2α knockout mice are pancytopenic with bone marrow hypocellularity and attenuated EPO levels; RBC production normalizes with the addition of recombinant EPO.[43] In addition, the postnatal deletion of HIF-2 abolishes EPO production by the kidney and liver, and overexpression of HIF-2 causes erythrocytosis.[44] HIF-2–mediated EPO transcription may require the cooperative binding of other transcription factors, such as hepatocyte nuclear factor 4, to the HREs.[45] HIF-1, on the other hand, is more important in regulating glycolysis, whereas some HIF-3 splice

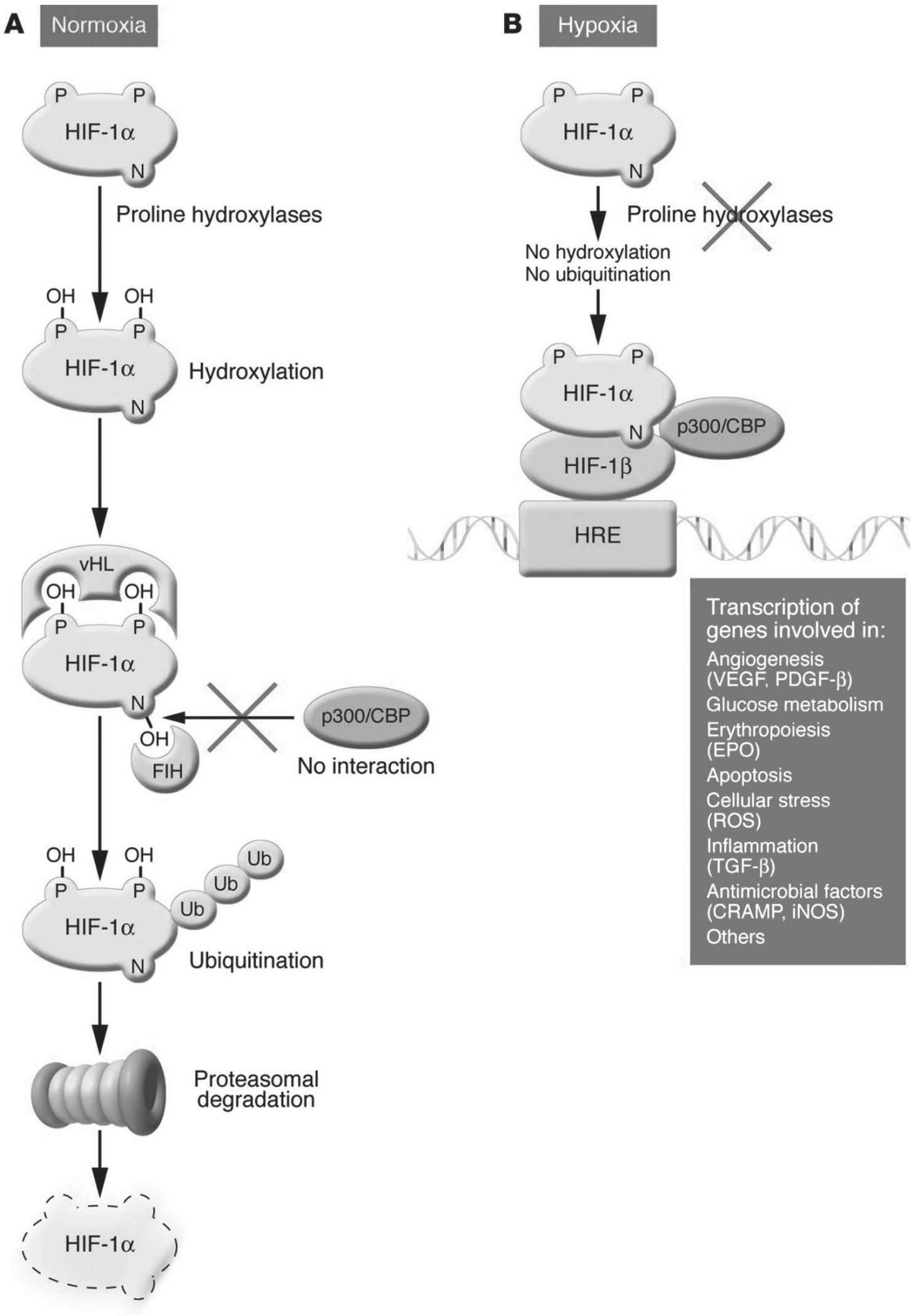

FIGURE 76.1 The regulation of hypoxia-inducible factor (HIF) expression. Under normoxic conditions **(A)**, proline hydroxylases (prolyl-4-hydroxylase domain proteins) target HIF for von Hippel-Lindau (vHL) protein binding, ubiquitination, and degradation. Under hypoxic conditions **(B)**, proline hydroxylases are inhibited, facilitating HIF-α heterodimerization and nuclear translocation, with consequent gene transcription. CBP, cAMP response element-binding protein; HRE, hypoxia response elements; VEGF, vascular endothelial growth factor; PDGF-β, platelet-derived growth factor-beta; ROS, reactive oxygen species; TGF-beta, transforming growth factor-beta; CRAMP, cathelin-related antimicrobial peptide; iNOS, inducible nitric oxide synthase. (Reproduced with permission from Zarember KA, Malech HL. HIF-1α: a master regulator of innate host defenses? *J Clin Invest*. 2005;115:1702.)

variants may actually inhibit transcription.[39] Interestingly, the HIF proteins activate the transcription of PHD-2 and PHD-3, thereby creating a negative feedback loop resulting in their own degradation.

EPO is a 165-amino acid (AA) glycoprotein hormone with a molecular weight of 30 kDa.[39] In adults, 90% of the EPO produced in response to anemia originates from the kidney, specifically from a population of cortical peritubular fibroblasts with neuronlike morphology in the inner cortex and the outer medulla, regions that tend to be especially susceptible to hypoxia.[46] EPO expression in other cell types is normally suppressed by transcription factors that bind to the tetranucleotide sequence, G-A-T-A, in the core promoter region of the gene (GATA transcription factors). Mild hypoxia, rather than increasing the transcription of EPO messenger RNA (mRNA), actually stimulates the brisk recruitment of previously quiescent clusters of cells within the kidney, each of which then generates EPO at a fixed rate. Moderate hypoxia, however, fuels additional EPO production by the liver, mainly from hepatocytes near the central veins and, to a lesser extent, from stellate or Ito cells.[39,46] EPO transcripts have also been isolated from bone marrow, the spleen, the brain, the lungs, hair follicles, and the reproductive tract, but are unlikely to contribute significantly to plasma EPO levels, because protein expression by these tissues has not been demonstrated.[20]

The erythropoietin receptor (EPO-R) is a 484-AA glycoprotein with a single membrane-spanning domain that homodimerizes during synthesis in the endoplasmic reticulum.[20,47] Each EPO-R binds to the tyrosine kinase, Janus kinase-2 (JAK-2), and the entire complex associates with the type 2 transferrin receptor (TfR-2) for trafficking to the cell membrane. TfR-2, a disulfide-bonded homodimer, is required to optimize the sensitivity of the EPO-R complex to circulating EPO and to efficiently synthesize growth differentiation factor (GDF)-15 (see the following).[47] Parenthetically, the Friend spleen focus-forming virus glycoprotein (gp55), a very different disulfide-bonded homodimer, can substitute for TfR-2 in facilitating EPO-R homodimerization and trafficking from the endoplasmic reticulum, resulting in EPO-independent EPO-R activation, autonomous erythroid proliferation, and leukemia in mice. Under normal conditions, the EPO-R:TfR-2 complex, upon binding EPO, undergoes a conformational change that allows its cytoplasmic domains to be tyrosine phosphorylated by JAK-2, stimulating signal transduction and activator of transcription (STAT)-5, mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI-3K), and protein kinase C (PKC).[39,48] The acute effects of EPO last only 30 to 60 minutes, after which JAK-2 and the EPO-R are dephosphorylated and the EPO:EPO-R complex may be internalized and degraded by the proteasome.[49] In addition, EPO also activates the phosphotyrosine phosphatase SHP-1, which binds to the EPO-R, dephosphorylates JAK-2, and terminates the propagation of the other intracellular signals. However, the primary signaling cascade set in motion by EPO-R activation is critical for the synthesis of erythrocytes.

Erythropoiesis takes place in the bone marrow, which is unquestionably the principal target of EPO activity. RBC production begins with the EPO-independent differentiation of the multipotent hematopoietic stem cell into the colony-forming unit granulocyte, erythrocyte, monocyte, megakaryocyte (CFU-GEMM), which in turn develops into the burst-forming unit erythroid (BFU-E).[50] The BFU-E is the first cell type in the erythroid lineage to express the EPO-R, and EPO is required for its survival and subsequent proliferation into several colony-forming units erythroid (CFU-E), a process that requires 10 to 13 days (Fig. 76.2). Of all the

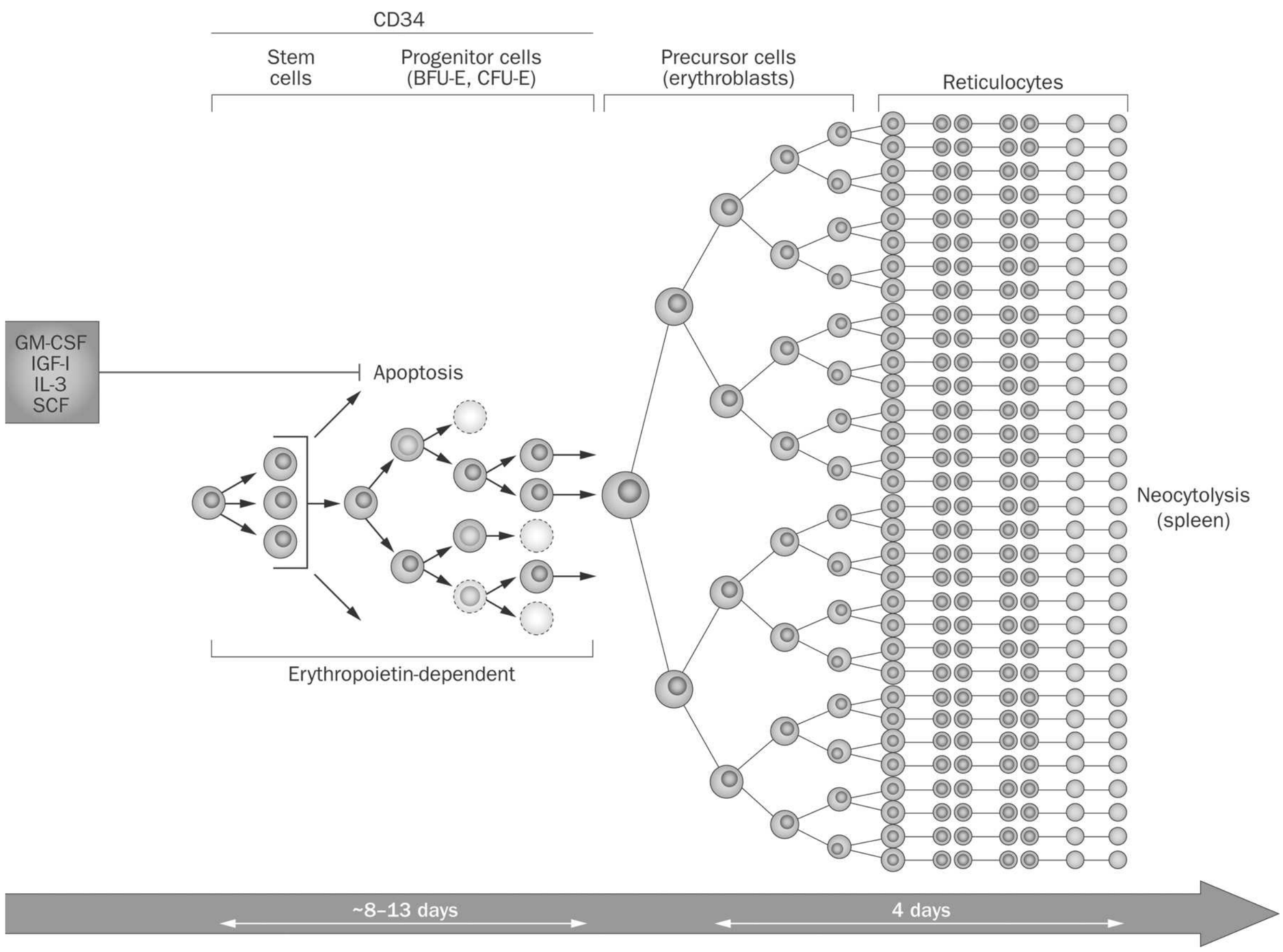

FIGURE 76.2 Normal erythropoiesis. The first stages of erythropoiesis, which require 8–13 days, involve the differentiation of the multipotent stem cell into the burst forming unit-erythroid (BFU-E) and colony forming unit-erythroid (CFU-E). These precursors require erythropoietin (EPO) and other growth factors to prevent apoptosis. The second stages of erythropoiesis, which require 4 days, involve the differentiation of erythroblasts to reticulocytes. Reticulocytes require EPO to avoid neocytolysis (premature destruction in the spleen). (Reproduced with permission from Besarab A, Coyne DW. Iron supplementation to treat anemia in patients with chronic kidney disease. *Nat Rev Nephrol.* 2010;6:699.)

erythroid precursors, the CFU-E has the highest membrane density of EPO-Rs, albeit < 1000 per cell, and additionally expresses TfR and GATA-1.[51] In the presence of EPO, GATA-1 promotes transcription of the antiapoptotic protein bcl-x_L, which allows the CFU-E to multiply. Conversely, in the absence of EPO, proapoptotic caspases are activated, and the CFU-E dies. A host of other growth factors, such as interleukin (IL)-3 and IL-9; granulocyte-colony stimulating factors and granulocyte, monocyte-colony stimulating factors (G-CSF and GM-CSF); and stem cell factors (SCFs) are also required for CFU-E proliferation.[50]

Subsequent phases of erythroid differentiation take place within erythroblastic islands.[51] Each erythroblastic island is a distinct, erythropoietic unit that consists of one central macrophage closely apposed to several maturing erythroblasts. Cell-specific molecular interactions, such as the binding between β1 integrins and vascular cell adhesion molecule (VCAM)-1, are critical to maintain the structure of these islands.[52] The CFU-E first differentiates into the proerythroblast, which again requires exposure to EPO to escape apoptosis. The proerythroblast has a large nucleus and expresses numerous membrane adhesion molecules such as CD44, intercellular adhesion molecule (ICAM)-1, and α4β1 and α5β1 integrins.[53] During maturation, the nucleus condenses and adhesion molecule expression is greatly diminished. Of note, CD44, the expression of which decreases by 30-fold during the progression from the proerythroblast to the orthochromatic normoblast, may be a more reliable marker of erythroid differentiation than the commonly used TfR, the expression of which remains relatively constant.

Terminal erythroid differentiation occurs as the erythroblast progresses through the basophilic, polychromatic, and orthochromatic normoblast stages. During this sequence, the cell acquires Hb, Rh factor, and the Duffy antigen, as well as skeletal proteins such as α- and β-spectrin, ankyrin, proteins 4.1R and 4.2, and the band 3 glycoprotein, which promotes membrane elasticity and stability.[53] The orthochromatic normoblast extrudes its pyknotic nucleus, which is ingested by the central macrophage and digested by deoxyribonuclease II, and becomes a reticulocyte.[51] This enucleated, multilobed reticulocyte is transformed into a biconcave erythrocyte during the next 2 to 3 days, initially in the bone marrow and then in the circulation, a process that involves membrane vesiculation, stabilization, and reorganization, as well as decreased cell volume. During erythrocyte maturation, cell membrane concentrations of aquaporin-1 (AQP-1), the glucose transporter (GLUT-4), the Na^+/H^+ exchanger (NHE-1), and the Na^+/K^+ pump are decreased, whereas the membrane skeletal proteins are retained.[54]

The Importance of Iron

Iron is essential for normal erythropoiesis. Total body iron is 50 mg per kilogram of body weight, or approximately 3500 mg for a 70-kg man.[55] This iron is distributed as about 65% in Hb; 10% in myoglobin (Mb), cytochromes, and enzymes; and the remainder in the reticuloendothelial system (RES), liver, and bone marrow. To meet the daily requirement of 300 billion fresh erythrocytes, differentiating erythroblasts need approximately 20 to 30 mg per day of iron, most of which is obtained from the recycling of senescent RBCs by phagocytic macrophages of the RES.[56] Heme from these cells is metabolized by heme oxygenase, and the Fe^{2+} released is sequestered by ferritin, which itself may undergo lysosomal degradation to hemosiderin.[55] The central macrophage of the erythroblastic island, acting as a "nurse cell," releases ferritin to its satellite erythroblasts, thus facilitating Hb synthesis and uninterrupted differentiation.[54]

Ferritin is indispensable for concentrating iron to the levels required for iron/oxygen chemistry, including aerobic metabolism, while avoiding the generation of insoluble ferric oxide and toxic oxygen radicals.[57] Ferritin is a hollow sphere with an external diameter of 12 nm and molecular weight of 480 kDa, consisting of 24 heavy and light polypeptide chains folded into four-helix bundles.[58] The center of the molecule's cavity has an internal diameter of ~7 to 8 nm, accessible via eight protein pores, which can accumulate up to 4000 atoms of iron. Iron must be reduced to Fe^{2+} to travel through the pores to the interior of the ferritin molecule, where it undergoes ferroxidase-catalyzed conversion to Fe^{3+} for storage.[57] Sequestration by ferritin increases the effective solubility of iron by 15 orders of magnitude.

Although only 1 to 2 mg per day of iron is obtained from the diet, gastrointestinal (GI) absorption of iron is required to offset minor losses from epithelial desquamation and microscopic bleeding.[56] The typical dietary intake of iron is 15 to 20 mg per day. Only 10% of dietary iron is present as relatively bioavailable heme compounds, which are readily absorbed into enterocytes and degraded by heme oxygenase to release Fe^{2+}. The remaining nonheme iron exists in the relatively unavailable Fe^{3+} state and must be reduced to Fe^{2+} by ferrireductase in conjunction with ascorbic acid; iron is then transported into the enterocyte by divalent metal transporter (DMT)-1.[55,59]

Cytosolic iron, whether present in duodenal enterocytes, macrophages, or hepatocytes, is delivered to the circulation via ferroportin (FP)-1, oxidized to Fe^{3+}, and bound to plasma transferrin.[60] In order to provide sufficient iron for reticulocyte production, transferrin-bound iron must be recycled 6 to 7 times daily.[7] Iron enters the erythroblast when two transferrin molecules bind TfR-1, which then undergoes endocytosis into a clathrin-coated siderosome.[55] The siderosome is acidified, releasing Fe^{3+} that is again reduced to Fe^{2+} by ferrireductase and exported to the cytoplasm via DMT-1. The apotransferrin:TfR-1 complex is recycled to the cell membrane and released into the circulation.[60] Meanwhile, cytosolic iron enters the mitochondrion, where ferrochelatase catalyzes its insertion into protoporphyrin IX to form heme, the critical component of Hb.[55,61]

Iron homeostasis is largely regulated at the level of GI absorption. One regulatory model postulates that plasma iron is sensed by duodenal crypt enterocytes via the TfR.[55] When iron is scarce, low cytosolic iron induces the transcription of

TfR-1, DMT-1, and FP-1 mRNA, all of which stimulate iron absorption. A second, compatible model proposes that iron absorption is downregulated by hepcidin, a 25-AA polypeptide produced by hepatocytes when iron is abundant. Hepcidin binds to FP-1 in enterocytes, macrophages, and hepatocytes themselves, promoting the JAK-2–mediated tyrosine phosphorylation, internalization, and degradation of FP-1; thus, hepcidin inhibits both the efflux of iron from the duodenum into the plasma as well as the mobilization of iron from the RES.[62] Hepcidin expression is itself regulated at critical points in the homeostatic loop. Specifically, hepcidin transcription is inhibited by HIF during tissue hypoxia, by soluble hemojuvelin during iron deficiency, and by EPO, GDF-15, and twisted gastrulation (TWSG-1) during erythroblast maturation. Conversely, hepcidin transcription is stimulated by iron-mediated production of bone morphogenetic proteins (BMPs), lipopolysaccharide, and IL-6 in states of systemic inflammation.[55,56,63,64] The biology of hepcidin is summarized in Figure 76.3.

A third model of iron regulation involves host defense pathways. Under conditions of infection or inflammation, macrophages generate DMT-1 and neutrophils release apolactoferrin and neutrophil gelatinase–associated lipocalin (NGAL), all of which remove iron from the circulation and decrease its availability to iron-dependent microorganisms.[60] NGAL is a 25-kDa protein, which similarly to hepcidin, sequesters iron during the acute phase response.[65] NGAL binds siderophores, high-affinity iron chelators produced by bacteria, and also functions as a nontransferrin pool of circulating iron.[66] During the acute phase response, cytokines such as IL-1β and tumor necrosis factor (TNF)-α also stimulate the translation of presynthesized ferritin subunit transcripts.[58] Presumably, this ferritin-mediated iron trapping is protective in states of acute inflammation, such as bacterial infection, but maladaptive in states of chronic inflammation, such as CKD.

Lastly, iron availability may regulate its own use via iron-regulatory elements (IREs). When cytosolic iron is abundant, transcription factors known as iron-regulatory proteins (IRPs) are rendered unable to bind their IRE; specifically, IRP-1 is converted into an aconitase and IRP-2 is degraded by the proteasome.[39,67] On the other hand, when iron is absent, IRPs bind to 3′- or 5′-regions of specific coding sequences, stimulating or inhibiting transcription, respectively. The IRP/IRE complex increases the transcription and translation of TfR-1 and DMT-1, promoting iron uptake into cells. Conversely, IRP/IRE decreases the expression of ferritin, which prevents iron sequestration; HIF-2α, which prevents EPO synthesis; and δ-aminolevulinic acid synthase 2, which prevents heme synthesis. Thus, scarcity of cytosolic iron blocks erythropoiesis and further iron consumption.[68]

Mechanisms of Anemia in Kidney Disease

The anemia of CKD and ESRD is the result of a confluence of many pathophysiologic processes. The most well-known of these is a deficiency of EPO relative to the degree of anemia. In subjects with normal Hb and kidney function, the EPO concentration is roughly 3 to 30 mU per milliliter,

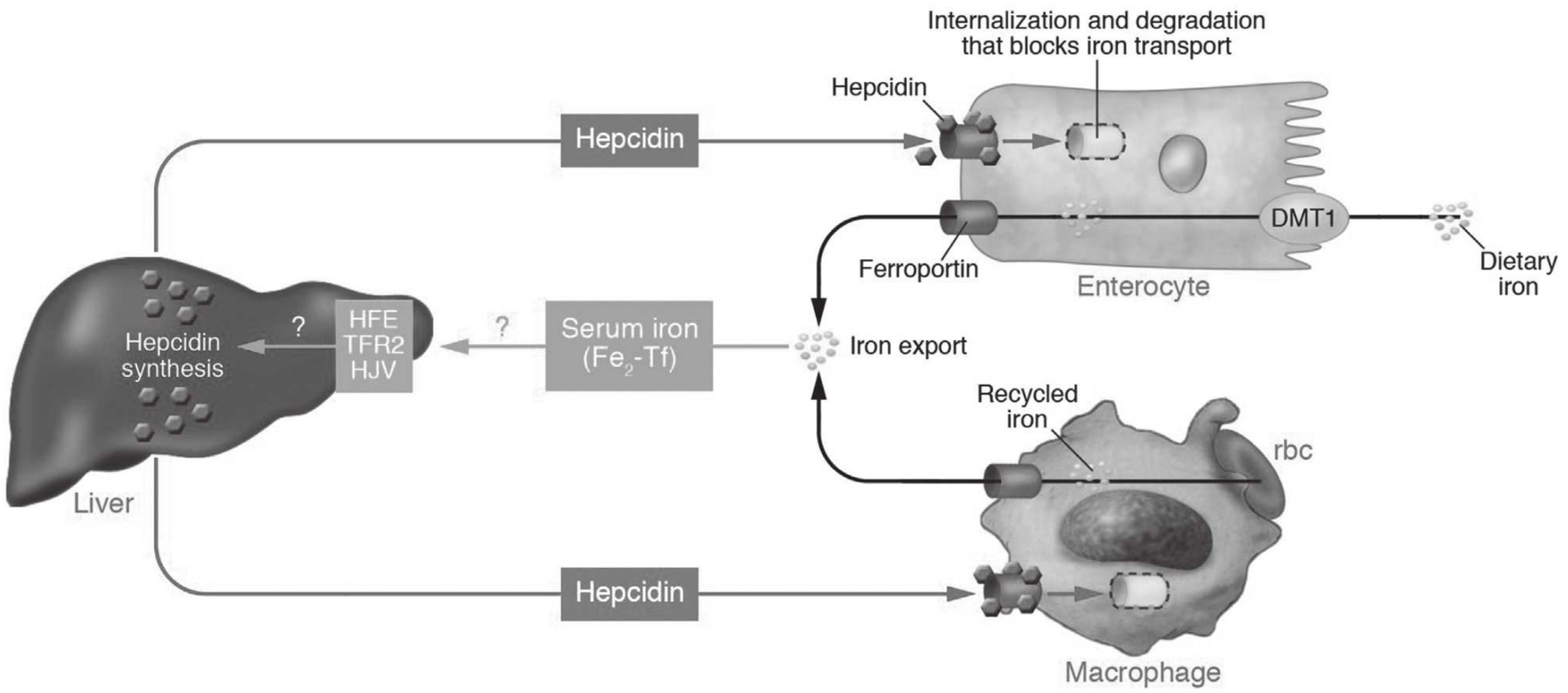

FIGURE 76.3 Hepcidin and iron homeostasis. Dietary iron uptake into the enterocyte involves transit via the divalent metal transporter 1 (DMT1). Iron recycled from senescent red blood cells (rbc) is stored in macrophages. Under conditions of iron sufficiency, increased diferric transferrin (Fe_2-Tf) is sensed by the human hemochromatosis (HFE) protein, type 2 transferrin receptor (TFR2), and the hemojuvelin (HJV) protein, and stimulates hepcidin production. Hepcidin binds to its ligand, ferroportin, and internalizes it back into the cytoplasm of enterocytes and macrophages, thereby sequestering iron and inhibiting its export from these cells. (Reproduced with permission from Vaulont S, Lou D-Q, Viatte L, et al. Of mice and men: the iron age. *J Clin Invest*. 2005;115:2079.) (See Color Plate.)

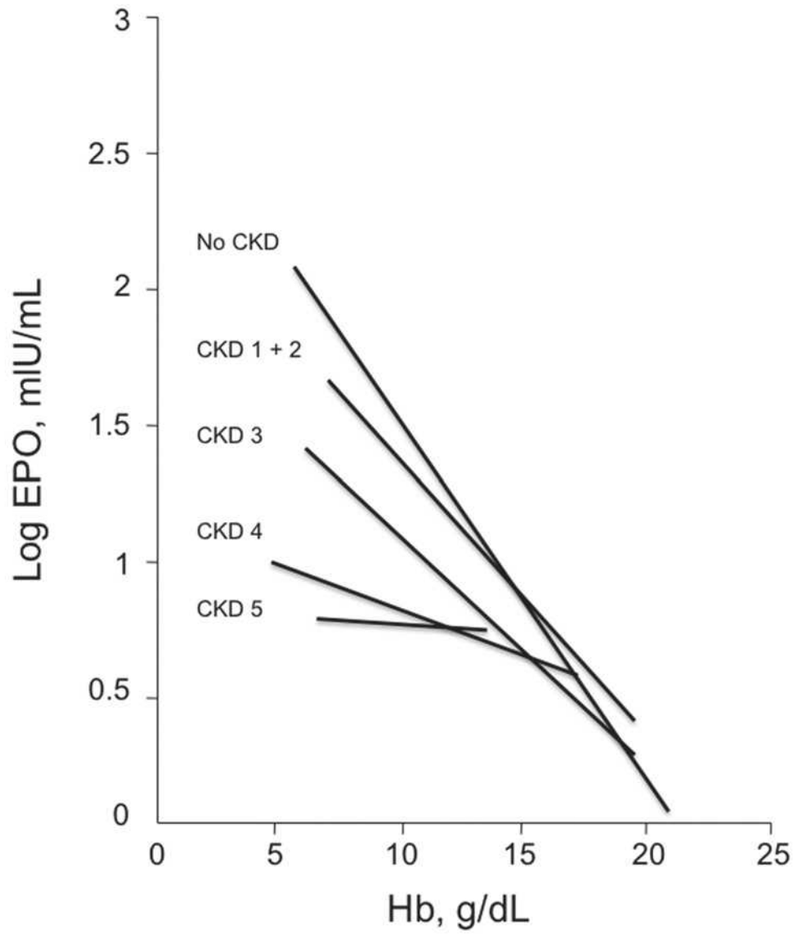

FIGURE 76.4 The relationship between hemoglobin (Hb) and log erythropoietin (EPO) concentrations. Each line represents the relationship for a specific stage of chronic kidney disease (CKD). (Reproduced with permission from Artunc F, Risler T. Serum erythropoietin concentrations and responses to anaemia in patients with or without chronic kidney disease. *Nephrol Dial Transplant*. 2007;22:2900.)

which increases by 100-fold as Hb falls.[69] In patients with kidney disease, the inverse relationship between EPO and Hb evaporates as kidney function declines, with little correlation at GFR or creatinine clearance (CrCl) < 30 to 40 mL per minute (Fig. 76.4).[69,70] In stages 4 through 5 CKD, this sluggish EPO response produces a normochromic, normocytic anemia.

Several mechanisms have been proposed to account for relative EPO deficiency in advanced CKD. (1) The diseased kidney adapts to an increased single-nephron sodium load by attenuating tubular sodium absorption. This change decreases O_2 consumption, improves oxygenation in the outer medulla, and reduces the stimulus for EPO production.[69] (2) EPO secreted into the circulation may be neutralized by soluble EPO-R, a 27-kDa splice variant identified in dialysis patients, the transcription of which is induced by inflammatory mediators, such as IL-6 and TNF-α.[71] (3) EPO may be inactivated by desialylation, a process mediated by proteases, the activity of which is increased in uremic patients.[72] (4) Even if EPO does reach the bone marrow intact and in sufficient quantity to stimulate erythropoiesis, its action may be blunted by the absence of permissive factors, such as IL-3, calcitriol, and CD4 cells, as well as by the presence of inhibitory factors, such as polyamines, ribonuclease, and parathyroid hormone (PTH).[72,73]

Iron deficiency, both absolute and relative, contributes significantly to anemia in kidney disease. Absolute iron deficiency is caused by blood loss, such as from repeated phlebotomy, colonic angiodysplasias, and occasionally, uremic bleeding. HD patients can also lose blood in the dialysis circuit, with cumulative iron losses averaging 1 to 3 g per year.[64] In addition, oral (PO) iron is poorly absorbed in advanced CKD, both because of hepcidin (see the following) as well as because of concomitantly prescribed medications such as proton pump inhibitors and calcium-, aluminum-, and lanthanum-containing compounds.[74]

Relative, or functional, iron deficiency occurs when the body is unable to mobilize otherwise adequate iron stores for erythropoiesis, a condition chiefly attributed to hepcidin. Hepcidin accumulates with progressive CKD, from 73 ng per milliliter in controls to 270 ng per milliliter in stages 2 through 4 CKD to 652 ng per mL in stage 5 CKD.[75] This increase probably results from a combination of persistent inflammation, frequent iron administration, and decreased renal clearance of hepcidin.[61,64] Interestingly, although anemia, hypoxia, the use of ESAs, and clearance by HD would be expected to decrease hepcidin levels, factors that induce hepcidin transcription clearly predominate. As previously mentioned, hepcidin binds to FP-1 and inhibits both the absorption of iron from the duodenum as well as the mobilization of iron from ferritin in the RES. NGAL, as well as hepcidin, is increased in HD patients and may contribute to functional iron deficiency.[76]

Iron deficiency is associated with reactive thrombocytosis in CKD and ESRD.[7] One mechanism to explain this effect involves the sequence homology between thrombopoietin and EPO.[77] As iron becomes scarce, EPO levels rise to compensate; in addition, patients may be given ESAs therapeutically. These high concentrations of either endogenous or exogenous hormone may accordingly stimulate platelet production. Thrombocytosis in response to iron deficiency, occasionally > 1,000,000 platelets per microliter, can be complicated by thromboembolic events, such as ischemic stroke, venous thromboembolism, central retinal vein occlusion, and cerebral venous sinus thrombosis.[78,79]

In patients with advanced CKD and ESRD, erythrocytes undergo accelerated destruction, a process that involves both young (neocytolysis) and old (senescence) RBC. Iron-poor, hypochromic cells are broken down during nadirs in EPO levels and undergo early phagocytosis because of abnormally high expression of phosphatidylserine.[80] The RBC cell membrane in HD patients is poorly deformable and lipid peroxidated, with unusual proportions of spectrin, ankyrin, protein 4.1, and band 3.[80,81] Many other changes in the composition of RBC membranes and intracellular machinery have been described in ESRD patients, involving AQP-1, the Na^+-K^+ pump, hexokinase, lactate dehydrogenase, and adenosine triphosphate (ATP), but the clinical significance of these abnormalities or their correction during dialysis is unknown.[82–84] Intravascular hemolysis can occur as a side effect of oxidizing medications, such as dapsone, primaquine, and nitrofurantoin. Hemolysis can also occur during HD because of mechanical lysis and exposure to water contaminants such as chloramines, nitrates, fluorine, arsenic, copper, and zinc.[85]

Nutritional deficiencies are easily overlooked causes of anemia in kidney disease. B-complex vitamins, specifically B_6 (pyridoxine), B_9 (folic acid), and B_{12} (cobalamin), are all essential cofactors in erythropoiesis. Deficiency of L-carnitine, a constituent of mitochondrial shuttle proteins involved in β-oxidation of fatty acids, occurs with the loss of kidney mass and via hemodialytic clearance. L-Carnitine supplementation induces heme oxygenase-1, activates the antiapoptotic protein Bcl-2, and increases CFU-E in animal cell cultures.[86,87] Hypophosphatemia, presumably via ATP depletion, impairs erythrocyte membrane deformability, leading to early senescence or even acute hemolytic anemia.[88] Moreover, scarcity of phosphorus decreases the availability of 2,3-bisphosphoglycerate for unloading O_2 from Hb, leading to tissue hypoxia.

DIAGNOSIS

As previously mentioned, the 2006 KDOQI guidelines advise an evaluation when Hb is < 13.5 g per deciliter in men and is < 12 g per deciliter in women, which are the mean Hb levels of the lowest 5th percentiles of the sex-specific adult population.[1] Hb is preferred over hematocrit (Hct) measurement, because the latter varies with glucose concentration, storage conditions, and analysis technique. Nonetheless, Hb measurements may vary by up to 0.5 g per deciliter within the same blood sample.[6] In CKD and PD patients, the timing of the Hb assessment is flexible. Conversely, in HD patients, Hb levels vary substantially during both the interdialytic and intradialytic periods, such that each 1 L of ultrafiltration increases Hb by 0.4 g per deciliter. Hb in these patients should be measured at the start of dialysis following a short (2-day) interdialytic interval (i.e., the midweek dialysis session). Hb measured at this time most closely approximates the mean weekly time-averaged Hb.[89]

The preliminary evaluation of anemia consists of a complete blood count (CBC), absolute reticulocyte count, ferritin, and either transferrin saturation (TSAT) or reticulocyte Hb content (CHr).[1] The CBC is useful for excluding aplastic anemia, myelodysplasia, or other bone marrow disorders (leukopenia, thrombocytopenia), folate or vitamin B_{12} deficiency (macrocytosis), and iron deficiency (microcytosis, hypochromia). The reticulocyte count, reticulocyte index, and reticulocyte production index estimate the erythropoietic response to anemia. The anemia of CKD and ESRD is nearly always hypoproliferative, especially at CrCl < 40 mL per minute. Even in this setting, EPO levels in most patients are within the normal range. For these reasons, the determination of EPO concentration in CKD and ESRD is of limited value.[69]

Iron status is a fundamental determinant of anemia, and iron deficiency can actually exist in the absence of anemia.[90] Fatigue, regardless of the Hb concentration, may be a prominent presenting symptom of iron deficiency because of the involvement of iron in enzymatic oxidative metabolism. The best parameter for assessing iron status remains elusive. RBC indices, such as low mean corpuscular Hb (MCH) or mean corpuscular volume (MCV) and a high red cell distribution width (RDW) often suggest iron deficiency but are nonspecific. A low RDW may be the earliest index of iron deficiency, but a low MCH may be more reliable.[90]

One commonly used test for iron deficiency is serum ferritin. Under normal conditions, 1 ng per milliliter of ferritin corresponds to approximately 8 mg of stored iron, but in states of chronic inflammation, serum ferritin is a poor marker of tissue ferritin, which is physiologically more significant but less easily measurable.[90] Serum ferritin can often reflect a leakage from tissue sources, is more highly glycosylated than the tissue form, and because it corresponds to molecular apoferritin, may contain little to no actual iron.[58,91] In CKD patients, a serum ferritin level < 10 to 30 ng per milliliter is associated with a fall in Hb, but levels of ferritin also correlate poorly with bone marrow iron (ferritin < 175 ng per milliliter, sensitivity and specificity to diagnose iron deficiency both 71%).[92,93] In HD patients, the relationship between ferritin and Hb is unclear, although ferritin levels < 500 ng per milliliter may more accurately reflect marrow iron scarcity.[94] Conversely, iron overload syndromes such as hemosiderosis are uncommon unless ferritin levels are > 2000 ng per milliliter.[58]

TSAT is calculated as serum iron divided by total iron binding capacity (TIBC) and represents the circulating iron available for erythropoiesis, specifically, iron-bound transferrin. Transferrin is a negative acute phase reactant, and in chronically inflamed CKD patients, TIBC is decreased from the normal mean of 330 to about 220 μg per deciliter.[7] TSAT varies diurnally, with maximal values occurring between 12:00 A.M. and 8:00 A.M. and minimal values (up to 58% less) occurring in the late afternoon and early evening.[95] This variability may confound an interpretation of TSAT unless the time of collection is standardized.[96] In the CKD population, Hb declines linearly with TSAT without a specific threshold, although action levels of 16% to 20% have been proposed.[1,92]

Because of these limitations, other assays have been advocated for the determination of iron status in patients with kidney disease. CHr measures the amount of Hb in reticulocytes, and provides a "snapshot" of iron available for erythropoiesis within the preceding 1 to 2 days.[96] CHr correlates reasonably well with TSAT, and threshold values of 29 to 32 pg per cell have been proposed to diagnose iron deficiency in HD patients. The reticulocyte Hb equivalent (RET-Hb), a related assay, facilitates the determination of CHr using commonly available blood analyzers.[97] In contrast, the percentage of hypochromic red blood cells (PHRC) measures the amount of Hb in the entire population of RBC, but suffers from inconsistency because RBC may sometimes expand during specimen storage.[96] Another test includes the measurement of soluble TfR, which is secreted by iron-hungry erythroblasts in states of iron deficiency in the presence of sufficient EPO or ESA. Increased TfR may be one of the first

76.1 A Comparison of Markers to Diagnose Iron Deficiency in Chronic Kidney Disease and End-Stage Renal Disease[7,58]

Marker	Diagnostic Threshold	Advantages	Disadvantages
Ferritin	< 100–200 ng/mL	Widely available	High variability, nonspecific if ↑
TSAT	< 20–25%	Widely available, ↑ sensitivity vs. ferritin	High variability
CHr	< 29–32 pg/cell	Accurate, inexpensive	Limited availability, ? diagnostic threshold
PHRC	< 6–10%	Accurate, inexpensive	Impractical with sample storage
sTfR	> 2.4 μg/mL	Accurate	Limited availability, ? diagnostic threshold
ZnPP	> 40–80 μmol/mol	Limited confounding by inflammation	Affected by lead concentration
Hepcidin	Unknown	Reflects functional iron deficiency	No reliable assay available
Bone marrow/ liver Bx	Unknown	Gold standard	Invasive, semiquantitative

TSAT, transferrin saturation; CHr, reticulocyte hemoglobin content; PHRC, percentage of hypochromic red blood cells; sTfR, soluble transferrin receptor; ZnPP, erythrocyte zinc protoporphyrin; Bx, biopsy.

signals of iron deficiency and is unaffected by inflammation.[98] In a recent study of HD patients, the soluble TfR-ferritin index had a higher predictive value for iron deficiency than either ferritin or TSAT alone.[99] Nonetheless, the diagnostic cut-off values of ferritin < 100 ng per milliliter, TSAT < 20%, and soluble TfR > 2.4 μg per milliliter appear to be poorly correlated.[98] High erythrocyte protoporphyrin and serum hepcidin concentrations have also been investigated as markers of iron deficiency, although an accurate measurement of the latter currently requires mass spectrometry and is thus impractical.[75,100] Table 76.1 lists some of the parameters that have been proposed to diagnose iron deficiency in CKD and ESRD.

TREATMENT

Therapy for the anemia of kidney disease has undergone a transformation during the past 5 to 10 years. ESAs remain a cornerstone of treatment, but successive randomized, controlled trials (RCTs) have demonstrated evidence of harm from using these agents to target higher Hb levels, leaving the nephrology community scrambling for alternate therapeutic agents. One such alternative is intravenous (IV) iron, various formulations of which improve Hb while concomitantly reducing doses of ESA. Moreover, there are several novel agents in active development that hold great promise for the treatment of anemia.

Erythropoiesis-Stimulating Agents

The U.S. Food and Drug Administration (FDA) first approved rHuEPO in 1989.[101,102] The first-generation ESAs include epoetin alfa (Epogen, Procrit, Eprex, Erypo, Espo) and beta (Neo-Recormon, Epogen), which are produced in Chinese hamster ovary cells, and epoetin omega (Epomax, Repotin), which is produced in baby hamster kidney cells. These compounds have the identical amino acid sequence as human EPO but differ in the degree of N- and O-linked glycosylation (Fig. 76.5). Conversely, epoetin delta derives from a human fibrosarcoma cell line, lacks the foreign N-glycolylneuraminic acid residues found in other rHuEPO congeners, and therefore more closely approximates the structure of endogenous EPO.[103] The second-generation ESAs, darbepoetin alfa (DPO, Aranesp) and methoxy polyethylene glycol-epoetin beta or continuous erythropoietin receptor activator (CERA, Mircera), are glycosylated in order to increase biologic half-life ($t_{½}$) and to decrease frequency of administration (see the following).[101,104] Although neither epoetin delta nor CERA are currently available because of financial and legal considerations, respectively, the development of newer, biosimilar ESAs continues in many countries worldwide.

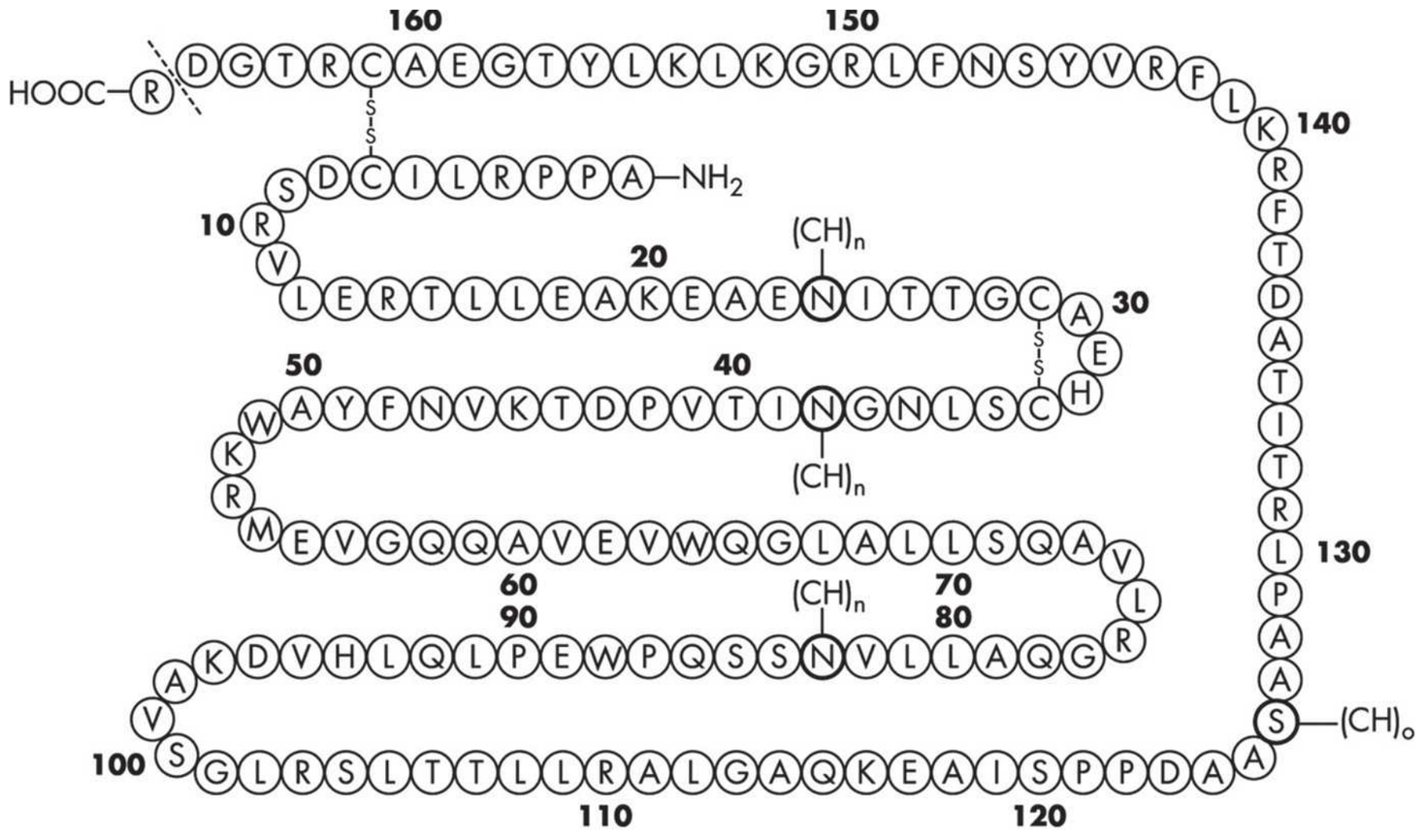

FIGURE 76.5 The amino acid sequence and structure of erythropoietin. $(CH)_n$ indicates *N*-linked glycosylation at asparagine residues 24, 38, and 83; $(CH)_o$ indicates *O*-linked glycosylation at serine residue 126. The C-terminal arginine at position 166 is cleaved prior to erythropoietin secretion. (Reproduced with permission from Ng T, Marx G, Littlewood T, et al. Recombinant erythropoietin in clinical practice. *Postgrad Med J.* 2003;79:367.)

The ESAs were approved on the basis of improved Hb and the avoidance of transfusions in small clinical trials, and observational studies suggested that ESA use was beneficial. In an adjusted analysis of nearly 90,000 Medicare ESRD patients, those who received ESAs most consistently 2 years before dialysis, as compared to those who received ESAs least consistently, had a 68% RR of 1-year mortality.[105,106] Furthermore, in a retrospective review of > 3000 patients undergoing maintenance dialysis, those who received epoetin beta had a 20% RR of 1-year mortality.[107] Recent systematic reviews and meta-analyses of studies in CKD and ESRD populations, prospective but of variable quality, have concluded that correcting anemia with ESAs is associated with improvements in left ventricular mass index (LVMI), a frequently cited surrogate for mortality, as well as both subjective and objective assessments of physical functioning and exercise tolerance.[18,108,109] A small trial, again in CKD and ESRD patients, has also demonstrated that the treatment of anemia with ESAs improves electrophysiologic markers of cognitive function.[110]

Notwithstanding the apparent benefits of ESA suggested by such studies, numerous large-scale RCTs have now compelled a belated reassessment of the hazards of aggressive ESA titration. Two landmark RCTs, the Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) and the Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin Beta (CREATE), were published in late 2006.[111–113] In CHOIR, 1432 patients with CKD stages 3 through 4 were treated with epoetin alfa to achieve Hb 11.3 or 13.5 g per deciliter. The trial was discontinued early because of a higher rate of a composite of death, stroke, myocardial infarction (MI), and hospitalization for CHF in the arm randomized to the higher Hb target. In CREATE, 603 patients with CKD stages 3 through 4 were treated with epoetin beta to achieve Hb 10.5 to 11.5 or 13 to 15 g per deciliter. This trial was completed and demonstrated a higher rate of dialysis initiation and hypertension (HTN) in the arm randomized to the higher Hb target. Most importantly, the Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT), which gained notoriety as the first placebo-controlled RCT of ESAs, was reported in 2009.[114] In TREAT, 4038 patients with type 2 diabetes and CKD stages 3 through 4 were treated either with DPO titrated to target Hb 13 g per deciliter, or with placebo, with rescue DPO given if Hb fell to < 9 g per deciliter. This study showed a higher rate of stroke, arterial and venous thromboembolic events, and cancer deaths in those with preexisting malignancy in the arm randomized to DPO. However, there were also improved measures of QOL and fewer transfusions in those assigned to active treatment.[114] The Normal Hematocrit trial, originally published in 1998 but recently updated with the release of supplemental data, previously reported comparable outcomes in HD patients. In the final analysis, 1265 patients on maintenance HD with CHF or ischemic heart disease were treated with epoetin alfa to achieve Hct 30% or 42%.[115] Again, the study was discontinued because of a trend toward a higher rate of death and nonfatal MI in the arm randomized to the higher Hct target.

Meta-analyses and systematic reviews of clinical trials involving ESA therapy in CKD and ESRD patients have yielded the same conclusions. One meta-analysis of nine RCTs, which included > 5000 patients with CKD, showed a higher risk of all-cause mortality with rHuEPO-targeted Hb of 12 to 16 g per deciliter (Fig. 76.6; RR 1.17, 95% confidence interval 1.01 to 1.35).[116] Also reported in this analysis and the accompanying editorial were a higher risk of HD access thrombosis, uncontrolled HTN, and MACE with more aggressive anemia correction.[117] A recent meta-analysis and a systematic review analyzed health-related QOL outcomes in 11 RCTs and showed no clinically meaningful differences in Short Form (SF)-36 scores, regardless of Hb levels achieved by ESA treatment.[118]

The route of administration of ESA has several important implications. Subcutaneous (SC) dosing of rHuEPO, compared to IV dosing, yields lower bioavailability (48.8%

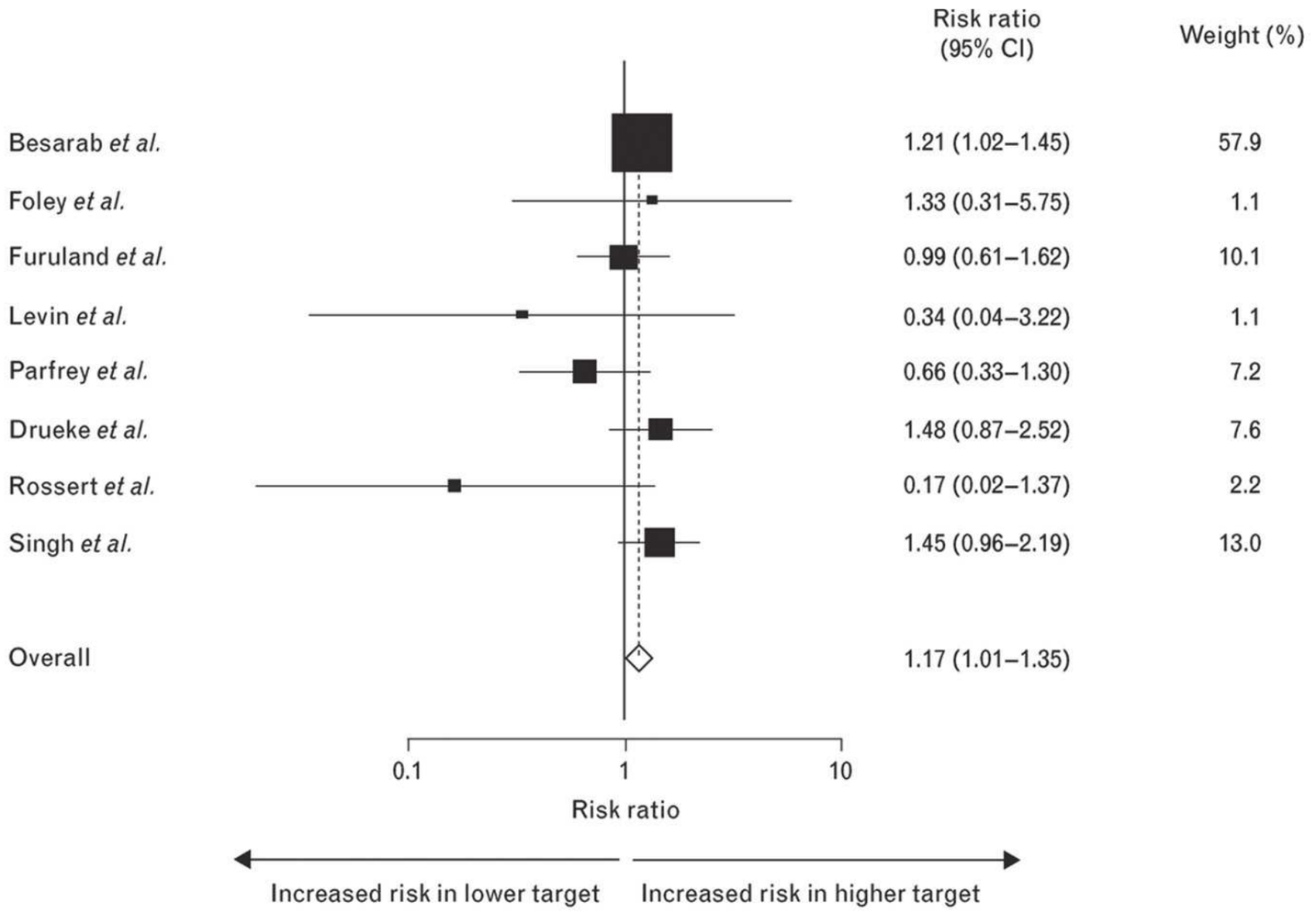

FIGURE 76.6 The risk of all-cause mortality in higher versus lower hemoglobin target groups in recent clinical trials (fixed effects analysis). (Reproduced with permission from Phrommintikul A, Haas SJ, Elsik M, et al. Mortality and target haemoglobin concentrations in anaemic patients with chronic kidney disease treated with erythropoietin: a meta-analysis. *Lancet*. 2007;369:381.)

and 100%, respectively) but higher and more sustained concentrations of the drug ($t_{½}$ = 19 to 25 hours and 5 to 11 hours, respectively).[119] Sustained levels of rHuEPO, within a critical range of 40 to 200 IU per kilogram, are required to trigger erythroid differentiation and to avoid neocytolysis, the process by which nascent RBCs undergo premature phagocytosis in the absence of circulating EPO. The SC administration of ESA, compared to the IV administration, thus may be more physiologic, and indeed causes less frequent dose-associated HTN. Because of the aforementioned pharmacokinetic differences, SC treatment, compared to IV treatment, also translates into a 14% to 32% decrease in dose and therefore cost. Hb levels may[120,121] or may not[122] be as stable with rHuEPO given SC dosing versus IV dosing. Because of logistical and patient compliance considerations, CKD and PD patients are often given DPO SC during clinic visits, whereas HD patients are currently given rHuEPO IV during dialysis.

Erythropoiesis-Stimulating Agent Hyporesponsiveness

Some patients with CKD and ESRD are unable to reach or maintain Hb levels of 11 to 12 g per deciliter, the range currently recommended by KDOQI, irrespective of ESA dose titration. This phenomenon is termed hyporesponsiveness, and has been defined as a continued need for rHuEPO at doses of > 300 IU/kg/week SC or > 400 to 450 IU/kg/week IV, or DPO at a dose of > 1.5 μg/kg/week SC (Table 76.2).[1,123] Inflammation, which is associated with a variety of clinical circumstances, is one of the main causes of ESA hyporesponsiveness.[124] The most familiar example of inflammation is the malnutrition, inflammation, atherosclerosis (MIA) or malnutrition-inflammation complex syndrome (MICS), which is found in 35% to 65% of HD patients. This syndrome is characterized by the presence of decreased Hb, transferrin, cholesterol, homocysteine, creatinine, and albumin and increased ferritin, IL-6, TNF-α, C-reactive protein (CRP), serum amyloid A, haptoglobin, and fibrinogen.[125] MICS is associated with lower QOL and higher rates of hospitalization and death, but its causes remain ambiguous. Potential etiologies include oxidative and carbonyl stress, loss of nutrients and antioxidants during dialysis, the accumulation of uremic toxins and inflammatory cytokines, and volume overload. In patients with MICS, the profusion of cytokines, particularly IL-1, IL-6, and TNF-α, inhibits endogenous EPO production and blocks the effect of rHuEPO on erythroid differentiation.[124]

Many clinical scenarios are associated with inflammatory ESA hyporesponsiveness. CKD patients with active malignancy, systemic lupus erythematosus (SLE), human immunodeficiency virus (HIV), or diabetic foot infections may have anemia that responds poorly to ESAs. CKD patients with chronic or acute decompensated CHF have increased levels of IL-1, IL-6, IL-18, TNF-α, endotoxins, aldosterone, angiotensin II, soluble adhesion molecules, and the soluble receptors TNFR-1, TNFR-2, IL-6R, and gp130, as well as volume overload, all of which may contribute to inflammation and anemia.[124,126] HD patients with periodontal disease often have an abnormal Hb, CRP, and erythrocyte sedimentation rate (ESR), the parameters of which improve with the treatment of disease.[127] HD patients who are underdialyzed (Kt/V < 1.33) show an association between dialysis adequacy and Hb, a finding that suggests that the elimination of uremic toxins is required to sustain adequate erythropoiesis.[128] Furthermore, HD patients who are exposed to endotoxin, impure dialysate, and bioincompatible membranes may have refractory anemia.[129] Patients who undergo HD via a tunneled, cuffed catheter (TCC) or an arteriovenous graft (AVG), compared to those who use an arteriovenous fistula

76.2 Causes of Erythropoiesis-Stimulating Agent Hyporesponsiveness

Causes	Examples
Inflammation	MICS, SLE, CHF, malignancy, failed kidney allograft Chronic infection: HIV, osteomyelitis, periodontal disease Hemodialysis-associated: impure dialysate, bioincompatible membranes, TCC/AVG use, inadequate dialysis
Iron restriction	Relative iron deficiency: inflammation Absolute iron deficiency: bleeding
Bone marrow disorders	Myelofibrosis, myelodysplastic syndrome Hemoglobinopathies: sickle cell anemia, thalassemia
Hemolysis	Oxidizing medications: dapsone, primaquine, nitrofurantoin Hemodialysis-associated: impure dialysate (e.g., chloramines, copper), mechanical destruction
Nutritional deficiencies	Pyridoxine, folic acid, cobalamin, L-carnitine, phosphorus
Miscellaneous	Pure red cell aplasia, severe SHPT, aluminum, ACEI/ARB

MICS, malnutrition-inflammation complex syndrome; SLE, systemic lupus erythematosus; CHF, congestive heart failure; HIV, human immunodeficiency virus; TCC, tunneled, cuffed catheter; AVG, arteriovenous graft; SHPT, secondary hyperparathyroidism; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

(AVF), are also more likely to show ESA hyporesponsiveness, presumably because of inflammation associated with a subclinical infection or the presence of foreign material in the vasculature.[130,131] Inflammatory ESA hyporesponsiveness has been treated with statins, vitamins C and E, oxpentifylline (also referred to as pentoxifylline), ultrapure water and biocompatible membranes in HD patients, and transplant nephrectomy in patients with failed allografts.[123]

Iron-restricted erythropoiesis is a common cause of ESA hyporesponsiveness. Functional iron deficiency, as previously discussed, occurs in patients with chronic inflammation, and is characterized by high ferritin and low TSAT, corresponding to the role of these biomarkers as acute phase and negative acute phase reactants, respectively. In 2005, 60% of HD patients had serum ferritins $>$ 500 ng per milliliter, and 22% of HD patients had serum ferritins $>$ 800 ng per milliliter.[124,132] Absolute iron deficiency, in contrast, occurs in patients with total body iron depletion, such as from GI bleeding, and is characterized by low ferritin and low TSAT. Iron supplementation may be beneficial in the treatment of both types of iron deficiency (see the following).

A rare but noteworthy cause of ESA hyporesponsiveness is pure red cell aplasia (PRCA), which occurs when patients previously exposed to ESAs develop anti-EPO antibodies.[133,134] Patients may be sensitized by treatment with any of the commercially available ESAs, although most cases have been reported in conjunction with a 2002 formulation of epoetin alfa (Eprex), in which albumin had been replaced by polysorbate-80. The neutralizing antibodies formed cross-react with rHuEPO as well as endogenous EPO, thus blocking activation of the EPO-R and subsequent erythropoiesis. Patients present with rapidly progressive anemia (Hb decreases by 0.1 g/dL/day), severe reticulocytopenia ($<$ 10,000 cells per microliter), and an absence of erythroid precursors on bone marrow biopsy. In addition, the failure of erythropoiesis results in a surfeit of iron (ferritin $>$ 1000 ng per milliliter and TSAT $>$ 70%). The detection of anti-EPO antibodies is necessary but not sufficient for a diagnosis of PRCA, because some patients with measurable antibody titers may not exhibit clinical symptomatology. PRCA is treated by withdrawing the ESA and prescribing corticosteroids with or without cyclosporine or cyclophosphamide.

Other causes of ESA hyporesponsiveness are also uncommon but may be valuable diagnostic considerations. Primary bone marrow disorders, such as myelofibrosis and myelodysplasia, as well as hemoglobinopathies, such as sickle cell anemia and thalassemia, are associated with a poor response to ESAs.[123] Secondary hyperparathyroidism is also linked to ESA-resistant anemia, possibly by causing increased fragility of RBCs, the inhibition of EPO synthesis and erythropoiesis, and the stimulation of bone marrow fibrosis. Small clinical trials show higher achieved Hb levels in response to a lower ESA dose after a parathyroidectomy.[135] Aluminum, by interfering with heme synthesis or iron bioavailability,

and angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), by blocking angiotensin II-mediated EPO release or hematopoietic stem cell maturation, are rarely reported causes of ESA hyporesponsiveness.[123,124,136] Hemolysis and nutrient deficiencies, as previously discussed, also contribute to ESA-refractory anemia.

Novel and Experimental Erythropoiesis-Stimulating Agents

New ESAs under development may have advantages over currently used agents, including a longer $t_{½}$, an absence of sequence homology with endogenous EPO, and in some cases, diverse mechanisms of action. CERA is synthesized by the integration of EPO with a 30-kDa methoxy polyethylene glycol polymer. This modification increases the $t_{½}$ from 8.5 hours with endogenous EPO to 130 hours with CERA.[103] CERA is administered either IV or SC every 2 to 4 weeks, compared to DPO, which is optimally given every 1 to 2 weeks, and rHuEPO, which is given every 2 to 3 days. Clinical trials have shown that CERA maintains stable Hb levels in both CKD and ESRD patients.[137,138] Other EPO-like molecules and derivatives, including synthetic erythropoiesis protein-polymers and fusion proteins in which EPO molecules are linked to flexible polypeptides, the Fc region of human IgG, or albumin have also been developed as means to enhance $t_{½}$.[103] Some of these compounds can be administered by ingestion, transdermal absorption, or aerosol inhalation.

Peginesatide (Hematide) is a pegylated, synthetic, dimeric peptide that activates the EPO-R but shares no sequence homology with endogenous EPO.[139] In vitro, peginesatide stimulates the proliferation and differentiation of erythroid progenitors, and in rodent and monkey models, the drug causes a dose-dependent rise in Hb levels. Because of its unique molecular structure, peginesatide should be unrecognizable to anti-EPO antibodies, and therefore could be used to treat PRCA. In an open-label trial involving 14 CKD patients with anti-EPO antibody-mediated PRCA, 28 months of peginesatide administration led to > 10-fold increase in reticulocyte counts, the suppression of anti-EPO antibody titers, a rise in Hb from median levels of 9 to 11.4 g per deciliter, and the elimination of transfusion requirements in most patients.[140] This molecule also has a $t_{½}$ of 14 to 60 hours, allowing monthly dosing in the same manner as CERA. Data from phase III clinical trials of peginesatide have been recently reported.[141,142] In PEARL 1 and 2,[141] a total of 983 CKD patients were randomized to either peginesatide or DPO to achieve Hb levels of 11 to 12 g per deciliter. These trials demonstrated the noninferiority of peginesatide to reach Hb targets, but a pooled analysis identified a higher risk of death, arrhythmia, and unstable angina in patients given peginesatide versus those given DPO (22% and 17%, respectively).[143] In EMERALD 1 and 2,[142] a total of 1626 HD patients were randomized to either peginesatide or rHuEPO to achieve Hb levels of 10 to 12 g per deciliter. These trials also demonstrated the noninferiority of peginesatide, but in this case showed no worrying safety signals. The discordance between the PEARL and EMERALD findings is currently unexplained.

HIF prolyl-hydroxylase inhibitors (PHIs) act in a manner mechanistically distinct from ESAs. The PHIs are orally active oxoglutarate analogs that inhibit PHD proteins, which are responsible for the hydroxylation, the ubiquitination, and the degradation of HIF-α, as discussed previously. These compounds mimic a hypoxic stimulus and lead to the stabilization of HIF-α, followed by heterodimerization, nuclear translocation, and HRE-mediated transcription of a multitude of genes. In fact, more than 100 genes are upregulated by PHIs, and the consequences of this pleiotropic activation are not yet fully understood.[103,144] Salutary effects include the stimulation of EPO release, the inhibition of hepcidin transcription, the amelioration of diabetic kidney disease, and protection from ischemia–reperfusion injury. Potentially deleterious effects include enhanced glycolysis, impaired mitochondrial respiration, aggravated tubulointerstitial injury, and increased vascular endothelial growth factor (VEGF)-mediated angiogenesis. In a small study, the administration of FG-2216, a first-generation PHI, increased EPO levels in six anephric ESRD patients from 7.8 to 240.6 mU per milliliter and in healthy controls from 6.4 to 81.2 mU per milliliter.[145] Although clinical trials of FG-2216 were halted following a case of fatal hepatic necrosis, studies with the related agent FG-4592 are ongoing and appear promising.

Additional therapeutic approaches to the anemia of CKD are the subject of active preclinical research. As discussed previously, some of the GATA transcription factors block EPO gene transcription, and GATA-2 inhibitors enhance HIF-1 activity, EPO levels, CFU-E and reticulocyte counts, and Hb concentration in a mouse model of anemia.[103,146] Separately, the phosphotyrosine phosphatase SHP-1, also known as hematopoietic cell phosphatase (HCP), binds to the src-homology 2 domain of the EPO-R, dephosphorylates JAK-2, and terminates the propagation of EPO-mediated signaling. HCP antisense oligonucleotide increases STAT-5 expression and BFU-E recovery in cells cultured from ESA-hyporesponsive HD patients.[147]

Iron Supplementation

Iron-restricted erythropoiesis, as previously mentioned, is common in CKD and ESRD patients. Correction of the iron deficiency should thus address one of the root causes of anemia and thereby reduce the dose of ESA required to maintain adequate Hb levels. In addition, iron supplementation may have nonhematologic benefits, such as improved cognition, thermoregulation, immune function, and exercise adaptation, as well as decreased restless legs syndrome and aluminum absorption.[148] The 2006 KDOQI guidelines recommend iron administration to maintain TSAT > 20% and ferritin 100 to 500 ng per milliliter in CKD and PD patients, and TSAT > 20% or CHr > 29 pg per cell and ferritin 200 to 500 ng per milliliter in HD patients.[1]

Recent evidence suggests that iron supplementation is beneficial in treating both absolute (ferritin < 100 to 200 ng per milliliter) and functional (ferritin > 100 to 200 ng per milliliter) iron deficiency. Specifically, the efficacy of IV iron in treating functional iron deficiency was evaluated in the Dialysis Patients' Response to IV Iron with Elevated Ferritin (DRIVE) trial.[112,149] In DRIVE, 134 HD patients with Hb levels < 11 g per deciliter, TSAT < 25%, and ferritin levels of 500 to 1,200 ng per milliliter were randomized to receive either ferric gluconate 125 mg IV during eight consecutive HD sessions (total dose, 1 g) or no iron. Patients with a malignancy or an active infection requiring systemic antibiotics were excluded. The EPO dose was ≥ 225 IU/kg/week or ≥ 22,500 IU per week at baseline and was increased by 25% at randomization. At 6 weeks, Hb increased by 1.6 ± 1.3 g per deciliter in the treatment group compared to 1.1 ± 1.4 g per deciliter in the control group (P = .028), with no difference in the rate of adverse events. TSAT and ferritin also increased in the patients given IV iron. DRIVE-II, a 6-week cohort follow-up of the original trial, showed that the rHuEPO dose fell by 7527 ± 18,021 IU per week in the treatment group compared to 649 ± 19,987 IU per week in the control group (P = .017).[150]

The choice between PO and IV iron therapy, especially in CKD patients, is somewhat controversial. PO iron includes the nonheme compounds, ferrous sulfate, chloride, fumarate, and gluconate, as well as the relatively new heme iron polypeptide (HIP).[74,151] Overdosing with PO iron is less likely than with IV iron, because hepcidin prevents GI absorption of excess iron in states of sufficiency (see previous). In advanced CKD, however, the unregulated excess of hepcidin and other acute phase and host defense molecules is precisely the cause of iron deficiency and iron-restricted erythropoiesis. Furthermore, the absorption of PO iron may be impaired by the concomitant ingestion of specific medications, and compliance with the increased pill burden is problematic. In four recent RCTs involving CKD patients, PO iron produced a lesser Hb response than IV iron.[152] Notwithstanding these limitations, PO iron is generally cost-effective, easily administered, and worth a trial of therapy before proceeding to IV infusion in CKD patients.

In contrast, PO iron is usually ineffective in HD and PD patients.[153] This poor response has been attributed to (1) dialysis-associated losses that exceed the amount of elemental iron absorbed orally, (2) increased time to maximal incorporation of PO iron into RBCs (33 days PO versus 8.6 days IV), and (3) high levels of hepcidin that block the duodenal uptake of PO iron.[74,154] A possible exception is HIP, which is absorbed from the GI tract 10 times more effectively than nonheme iron. A 6-month trial of HIP in maintenance HD patients concluded that PO heme iron successfully replaced IV iron and was associated with increased rHuEPO efficiency.[151] The oral HEMe iron polypeptide Against Treatment with Oral Controlled Release Iron Tablets (HEMATOCRIT) trial, a 6-month study of HIP in PD patients, is currently under way.[155]

A common clinical conundrum in ESRD patients is whether to prescribe iron in low doses at regular intervals (maintenance dosing) or in high doses after iron deficiency develops (repletion dosing). Repletion dosing, which consists of ≥ 1 g of iron administered during 1 to 8 infusions, is given when TSAT is < 20% to 25% or ferritin levels are < 100 to 200 ng per milliliter. This dose is considered an adequate test of iron responsiveness, which is assessed by the serial measurement of iron indices and Hb.[74] Maintenance dosing, which consists of 22 to 65 mg of iron administered weekly, may be advantageous in minimizing Hb variability, maximizing ESA sensitivity, and overcoming ESA hyporesponsiveness.[156,157] This approach is recommended by the most current KDOQI guidelines.[1]

Intravenous Iron: Options and Toxicity

Many formulations of IV iron are available, and are useful in both CKD and ESRD patients (Table 76.3). The iron dextrans are formulated as high molecular weight ([HMW] 265 kDa; Imferon, DexFerrum) and low molecular weight ([LMW] 165 kDa; INFeD) compounds. Because of the association of the HMW agents with anaphylactoid reactions, Imferon has been withdrawn from the market, and DexFerrum is increasingly avoided. The risk of adverse events, including life-threatening episodes, is much less with LMW (< 1 in 200,000 doses) than with HMW iron dextran.[148,158,159] Iron dextran has been given in doses of 2 to 3 g within 4 to 10 minutes, although in clinical practice, doses of 1 to 3 g are infused during the course of a 4-hour HD session. Advantages of LMW iron dextran include providing ≥ 1 g of iron in a single infusion and saving an estimated $250 million yearly when used instead of more expensive preparations. Conversely, the preferential use of proprietary IV iron formulations to avoid the rare adverse events associated with LMW iron dextran would cost $7.8 million USD to prevent a single life-threatening event and $33 million USD to prevent a single death.[148,159]

IV iron is also manufactured as iron sucrose (Venofer) and ferric gluconate (Ferrlecit), which is available as a generic equivalent (Watson Pharmaceuticals, Corona, CA). Iron sucrose is delivered at doses ≤ 200 to 300 mg during a 2-hour infusion, because single doses of 400 to 500 mg have been linked to infusion-associated hypotension and coronary vasospasm.[160,161] Ferric gluconate is delivered at doses of 125 to 250 mg during a 1- to 4-hour infusion; again, doses exceeding this range have been linked to hypotension. Both agents thus require four to eight sequential infusions to achieve a cumulative dose of 1 g of elemental iron. Ferric gluconate, compared to iron sucrose and iron dextran, donates iron most readily to apotransferrin, a property that should theoretically increase the efficiency of erythropoiesis and should decrease the oxidative stress provoked by free iron, although the clinical relevance of these observations is unknown.[154,162] On a separate note, small studies in CKD patients have shown consistently that albuminuria, enzymuria, and the excretion of N-acetyl-β-glucosaminidase (NAG) increase following the

76.3 Currently Available and Investigational Intravenous Iron Formulations[148,175,177]

Generic Name	Trade Name	Standard Dose	Infusion Rate	WAC (USD)
LMW iron dextran	INFeD	1–3 g	2–4 hours	$24.10 (100 mg/vial)
HMW iron dextran	DexFerrum	1–3 g	2–4 hours	$36.00 (100 mg/vial)
Iron sucrose	Venofer	200–300 mg	2–3 hours	$55.00 (100 mg/vial)
Ferric gluconate	Ferrlecit	125–250 mg	1–4 hours	$31.80 (62.5 mg/ampule)
Ferumoxytol	Feraheme	510 mg	17 seconds	$396.78 (510 mg/vial)
Ferric carboxymaltose	Ferinject	750–1,000 mg	15 minutes	N/A
Iron isomaltoside 1000	Monofer	1.5–1.7 g	1 hour	N/A

WAC, wholesale acquisition cost (as of March 28, 2011); USD, United States dollars; LMW, low molecular weight; HMW, high molecular weight; N/A, not available.

administration of iron sucrose but not ferric gluconate.[163–165] Iron sucrose, compared to ferric gluconate and iron dextran, causes the most severe injury to proximal tubule and endothelial cells in vitro, although all three agents induce significant lipid peroxidation.[166] This result has been recapitulated in HD patients, in whom the infusion of iron sucrose caused increased peroxidation of polyunsaturated fatty acids as measured by the generation of malondialdehyde.[167]

Additional concerns have surfaced about the use of IV iron, regardless of the formulation. The possibility of iron overload and hemosiderosis has caused considerable anxiety. Before the ESA era, repeated blood transfusions led to more frequent parenchymal iron deposition, but serum ferritin levels in these cases were often > 5000 ng per milliliter.[74] The risk of iron overload, which begins when total body iron is > 5 g (the capacity of the RES), is low as long as ferritin levels are < 2000 ng per milliliter. A hypothetical risk of IV iron is the acceleration of systemic infection by the suppression of phagocytosis and the provenance of iron to microorganisms.[148] Clinical data underpinning this fear are limited, and both prospective and retrospective clinical studies have failed to link bacteremia to the use of IV iron.[168,169]

Novel and Experimental Iron Agents

The newer PO iron compounds have been designed to have better bioavailability than their older congeners. HIP was discussed previously. Soluble ferric pyrophosphate (SFP) has been found, in vitro, to increase ferritin production compared to ferric sulfate and chloride, with less sensitivity to inhibition by divalent cations.[170] Furthermore, an emulsification of ferric pyrophosphate within albumin microspheres is superior to SFP, as assessed by total absorption from the GI tract in a rat model.[171] Low doses of sodium feredetate, a different formulation, have proven more effective than high doses of ferric fumarate in pregnant, anemic women.[172] Conversely, the efficacy of iron hydroxide polymaltose in treating iron-deficiency anemia has been repeatedly questioned.[173] The value of any of these medications in treating CKD and ESRD patients is unknown.

One of the newest formulations of IV iron is ferumoxytol (Feraheme), a carbohydrate-coated, superparamagnetic iron oxide nanoparticle.[174] The chief advantage of ferumoxytol is the ability to give a 510-mg dose by IV push in as little as 17 seconds without adverse effects. This speedy delivery is possible because of the absence of free iron in the formulation, and is clearly an attractive alternative to prolonged IV infusions in ambulatory CKD and PD patients. In RCTs involving CKD and HD patients, two doses of ferumoxytol 510 mg IV were more effective than 3 weeks of ferrous fumarate 200 mg per day PO, with the rare occurrence of anaphylactoid and hypersensitivity reactions and hypotension. Ferumoxytol may also affect magnetic resonance imaging (MRI) studies for up to 3 months after administration.

Ferric carboxymaltose (Ferinject) is a carbohydrate shell–stabilized ferric hydroxide core.[175] Ferric carboxymaltose also contains little free iron and can be given as a 1-g dose in ≤ 15 minutes. In RCTs involving CKD and ESRD patients, ferric carboxymaltose 1 g IV was more effective than ferric sulfate 65 mg three times per day PO and as effective as equivalent doses of iron sucrose, as assessed by improvements in TSAT, ferritin, Hb, and health-related QOL. Adverse events with ferric carboxymaltose were rare and less frequent than with iron sucrose. The Randomized Evaluation of Efficacy and Safety of Ferric Carboxymaltose in Patients with Iron Deficiency Anemia and Impaired Renal Function (REPAIR-IDA) trial will randomize CKD patients to two 750-mg doses of ferric carboxymaltose versus five 200-mg doses of iron sucrose.[176]

Iron isomaltoside 1000 (Monofer) is the latest addition to the IV iron armamentarium and may possess some unique advantages. Iron isomaltoside 1000 can be given in doses of up to 20 mg per kilogram of body weight and is relatively pure. In contrast, ferric carboxymaltose is limited to doses of 15 mg per kilogram of body weight and contains up to 75 μg per milliliter of aluminum and 5.5 mg per milliliter of sodium, the impurities of which may be problematic for patients with kidney disease.[177] In an open-label trial involving 182 CKD and ESRD patients, iron isomaltoside 1000, administered as either bolus injections of 100 to 200 mg or as a fast high-dose infusion, resulted in increased TSAT, ferritin, and Hb, with no immediate or delayed allergic reactions.[178]

CONCLUSION

Anemia is common in the CKD population and is associated with increased morbidity and mortality. The anemia of CKD has its origins within a confluence of pathobiologic circumstances that include relative EPO and iron deficiencies. Unraveling its etiologic and diagnostic characteristics remains challenging. During the course of modern history, the management of anemia in patients with kidney disease has progressed from the archaic, involving whole blood transfusions, to the contemporary, encompassing evidence-based and Hb-targeted ESA and iron administration, and continues to evolve with the discovery of new mechanisms and therapeutic agents. As scientific inquiry proceeds, we anticipate significant advances in the treatment of this complex condition, the anemia of chronic kidney disease.

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