

Mechanisms of Anemia in CKD

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ABSTRACT

Anemia is a common feature of CKD associated with poor outcomes. The current management of patients with anemia in CKD is controversial, with recent clinical trials demonstrating increased morbidity and mortality related to erythropoiesis stimulating agents. Here, we examine recent insights into the molecular mechanisms underlying anemia of CKD. These insights hold promise for the development of new diagnostic tests and therapies that directly target the pathophysiologic processes underlying this form of anemia.

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Anemia was first linked to CKD over 170 years ago by Richard Bright.¹ As kidney disease progresses, anemia increases in prevalence, affecting nearly all patients with stage 5 CKD.² Anemia in CKD is associated with reduced quality of life and increased cardiovascular disease, hospitalizations, cognitive impairment, and mortality.²

Anemia in CKD is typically normocytic, normochromic, and hypoproliferative. The demonstration of a circulating factor responsible for stimulating erythropoiesis, and the kidney as the main source of erythropoietin (EPO) in the 1950s^{3,4} engendered the hypothesis that EPO deficiency is a predominant cause of anemia in CKD. Purification and cloning of EPO in the late 1970s and 1980s^{5–7} enabled the development of immunologic assays for quantitating levels of circulating EPO. Although generally normal or slightly increased in anemia of CKD, EPO levels are considered inappropriately low relative to the degree of anemia, because similarly anemic patients with normal kidney function have 10–100 times higher EPO levels.^{8–10} One important limitation of such assays is that they measure all

immunogenic EPO fragments, which do not all correlate with biologic activity.^{11,12}

Anemia management was revolutionized in the late 1980s with the introduction of recombinant human EPO. This and related erythropoiesis stimulating agents (ESAs) greatly benefited patients by improving their debilitating symptoms, and freeing them from dependence on blood transfusions with their associated complications (secondary iron overload, infections, and sensitization impeding transplantation).^{2,13–15} However, even in the initial studies, adverse effects were noted in patients receiving ESAs, including worsening hypertension, seizures, and dialysis access clotting.^{14,15} In addition, ESAs do not reduce adverse outcomes associated with anemia, such as mortality, nonfatal cardiovascular events, left ventricular hypertrophy, hospitalizations, and progression of kidney disease, in prospective randomized controlled trials.² In fact, recent trials in both hemodialysis and predialysis CKD patients demonstrate an increased risk of death, adverse cardiovascular events, and stroke by administering ESAs to target hemoglobin levels >11 g/dl.^{16–19} Secondary

analyses of these studies suggest that elevated hemoglobin *per se* does not confer the increased risk, but rather higher doses of ESAs and relative resistance to ESAs, although this has not been studied directly.^{20,21} In addition, ESAs have been associated with increased progression of malignancy and death in cancer patients.²²

Why would ESAs have these adverse effects? Although relative EPO deficiency may contribute to the anemia of CKD,²³ it is not the sole cause. Indeed, anemia of CKD is resistant to ESAs in approximately 10%–20% of patients.² It seems likely that supraphysiologic doses of ESAs, especially at very high doses or in patients resistant to treatment, have off-target effects in other tissues. These findings have renewed interest in understanding the molecular mechanisms of anemia in CKD, with the hope of developing new therapies that more closely target the underlying pathophysiology of low hemoglobin.

Aside from EPO deficiency, what else contributes to the anemia of CKD? Numerous studies suggest that circulating uremic-induced inhibitors of erythropoiesis contribute to the anemia, although this has been disputed in some

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studies and no specific inhibitors have been identified.^{13,23} Shortened red blood cell survival also contributes, as demonstrated by radioisotope labeling studies.^{23–25} Although the etiology is not entirely clear, metabolic and mechanical factors have been proposed.^{23,24} Nutritional deficiencies, such as folate and vitamin B₁₂, due to anorexia or dialysate losses are currently uncommon with the routine use of supplementation in hemodialysis patients.²³ Whereas hemodialysis patients historically developed secondary iron overload from recurrent blood transfusions, the modern era of ESA treatment has uncovered an increasingly recognized role for disordered iron homeostasis as a major contributor to the anemia of CKD.

Based on its ability to donate and accept electrons, iron is essential for many important biologic reactions, including oxygen transport, cellular respiration, and DNA synthesis. However, this same property makes excess iron toxic by generating free radicals that can damage or destroy cells. Systemic and cellular iron levels must therefore be tightly regulated. The majority of iron (20–25 mg) is provided by recycling from senescent red blood cells, which are phagocytosed by reticuloendothelial macrophages to store iron until it is needed, with lesser amounts provided by dietary absorption in the duodenum (1–2 mg) and release from liver stores. Plasma iron, which circulates bound to transferrin, is relatively limited at 3 mg, and therefore must be turned over several times to meet the daily requirements for erythropoiesis. With no regulated mechanism for iron removal, typical iron losses are 1–2 mg daily, mainly from intestinal and skin cell shedding and menstruation in reproductive-age women. Systemic iron balance is therefore maintained by regulating dietary iron absorption and iron release from storage sites in the liver and reticuloendothelial macrophages.²⁶

CKD patients have increased iron losses, estimated at 1–3 g per year in hemodialysis patients, due to chronic bleeding from uremia-associated platelet dysfunction, frequent phlebotomy, and blood trapping in the dialysis apparatus.²³ CKD patients, particularly

hemodialysis patients, also have impaired dietary iron absorption. Indeed, oral iron was no better than placebo and was less effective than intravenous iron at improving anemia, improving or preventing iron deficiency, or reducing ESA dose in hemodialysis patients.^{27–29} In addition, many CKD patients receive ESAs, which deplete the circulating iron pool by increasing erythropoiesis. Thus, CKD patients are prone to true iron deficiency, and iron supplementation is part of mainstay of anemia treatment in CKD. Intravenous iron is preferred for hemodialysis patients because of impaired dietary iron absorption.²

In addition to true iron deficiency, many CKD patients have functional iron deficiency, characterized by impaired iron release from body stores that is unable to meet the demand for erythropoiesis (also called reticuloendothelial cell iron blockade). These patients have low serum transferrin saturation (a measure of circulating iron) and normal or high serum ferritin (a marker of body iron stores). Some of these patients are treated with intravenous iron, a trend that seems to be increasing with the recent controversy surrounding ESAs. However, for patients with high serum ferritin ≥ 500 –800 ng/ml, management is less clear.² Concerns about treating these patients with iron include poor effectiveness and the potential for adverse effects, including oxidant-mediated tissue injury from excess iron deposition and increased risk of infection. One limitation is that high serum ferritin is not specific for increased body iron stores because ferritin is also affected by infection, inflammation, liver disease, and malignancy.²

Recent data suggest that hepcidin excess may account for the impaired dietary iron absorption and reticuloendothelial cell iron blockade present in many CKD patients. Discovered independently by three groups in 2000–2001,^{30–32} hepcidin is the main hormone responsible for maintaining systemic iron homeostasis.²⁶ Produced by the liver and secreted into circulation,^{30–32} hepcidin binds and induces degradation of the iron exporter, ferroportin, on duodenal enterocytes,

reticuloendothelial macrophages, and hepatocytes to inhibit iron entry into the plasma.³³ Inflammatory cytokines directly induce hepcidin transcription,²⁶ presumably as a mechanism to sequester iron from invading pathogens, leading to the iron sequestration, hypoferrinemia, and anemia that are hallmarks of many chronic diseases including CKD.

The development of assays to measure bioactive hepcidin in the last 2–3 years has ignited a profusion of studies investigating the role of hepcidin excess in the anemia of CKD. Numerous studies now show that hepcidin is elevated in CKD patients.^{26,34–36} Mechanisms suggested to account for this are increased expression by inflammatory cytokines and reduced renal clearance.^{26,34–36} Studies are ongoing to determine whether hepcidin measurement will have diagnostic utility in CKD patients regarding iron status, inflammatory status, or ESA responsiveness or resistance. Complicating factors are the lack of uniformity in hepcidin measurements by different assays,³⁷ and the complex interplay of various factors that influence hepcidin levels in CKD patients, including iron, inflammation, and reduced renal clearance that tend to increase hepcidin, and anemia, ESAs, dialysis clearance, and hypoxia that tend to reduce hepcidin.²⁶

Recognition of a key role for hepcidin excess in causing the functional iron deficiency and anemia of CKD has ignited interest in targeting the hepcidin-ferroportin axis as a new treatment strategy for this disease. By blocking hepcidin and/or increasing ferroportin activity, these agents could improve dietary iron absorption and iron mobilization from the patients' own body stores, thereby minimizing the need for supraphysiologic doses of intravenous iron and ESAs with their potential adverse effects. Importantly, in CKD patients with hepcidin excess, large intravenous boluses of iron would be predicted to have limited effectiveness because much of the iron is rapidly taken up by the liver and sequestered, and the remainder that is incorporated into red blood cells would be recycled ineffectively. In addition, intravenous iron itself would further increase in hepcidin

levels³⁵ and worsen this phenomenon. Several strategies under investigation include antagonizing hepcidin directly, inhibiting hepcidin production, interfering with the hepcidin/ferroportin interaction, or stabilizing ferroportin.^{38–41} Initial small studies suggest that targeting this pathway can increase iron mobilization and hemoglobin in animal models of anemia of chronic disease.^{39–41} The side effect profiles and whether these strategies will prove effective for treating anemia of CKD in humans remain unknown.

In summary, anemia of CKD is a multifactorial process due to relative EPO deficiency, uremic-induced inhibitors of erythropoiesis, shortened erythrocyte survival, and disordered iron homeostasis. Recent work has identified hepcidin excess as a main contributor to the disordered iron homeostasis and anemia of CKD by impairing dietary iron absorption and iron mobilization from body stores (Figure 1). Improving our understanding

of the molecular mechanisms underlying anemia of CKD holds promise for developing new pharmacologic agents that more closely target the underlying pathogenic mechanisms of this disease for improved efficacy and reduced treatment-related adverse outcomes.

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DISCLOSURES

J.L.B. and H.Y.L. have ownership interest in a startup company, Ferrumax Pharmaceuticals Inc., which has licensed technology from the Massachu-

setts General Hospital based on work cited here and described in prior publications.

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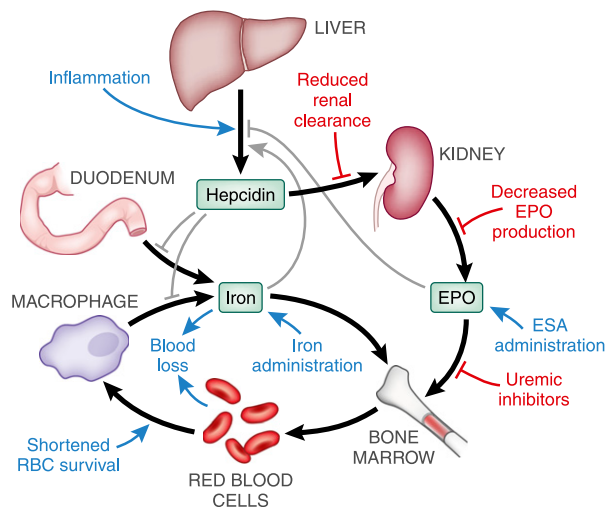


Figure 1. Schematic representation of the mechanisms underlying anemia of CKD. Iron and EPO are crucial for red blood cell production in the bone marrow. Iron availability is controlled by the liver hormone hepcidin, which regulates dietary iron absorption and macrophage iron recycling from senescent red blood cells. There are several feedback loops that control hepcidin levels, including iron and EPO. In CKD patients (particularly in end stage kidney disease patients on hemodialysis), hepcidin levels have been found to be highly elevated, presumably due to reduced renal clearance and induction by inflammation, leading to iron-restricted erythropoiesis. CKD also inhibits EPO production by the kidney, and may also lead to circulating uremic-induced inhibitors of erythropoiesis, shortened red blood cell lifespan, and increased blood loss. Black and gray arrows represent normal physiology (black for iron and hormonal fluxes, gray for regulatory processes). Colored arrows represent the additional effects of CKD (blue for activation, red for inhibition). RBC, red blood cell.

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