CME Iron deficiency syndromes and iron-restricted erythropoiesis

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The relationships between erythropoietin (EPO), iron, and erythropoiesis and the presence of iron-restricted erythropoiesis have important implications in anemia management. Iron-restricted erythropoiesis occurs in the presence of one or more iron deficiency syndromes: absolute iron deficiency, functional iron deficiency, and/or iron sequestration. Absolute iron deficiency is a common nutritional deficiency in women's health, pediatrics, and the elderly and is therefore an important public health problem. Functional iron deficiency occurs in patients with significant EPO-mediated erythropoiesis or therapy with erythropoiesis-stimulating agents, even when storage iron is present. Iron sequestration mediated by hepcidin is an underappreciated but common cause of iron-restricted erythropoiesis in patients with chronic inflammatory disease. The challenge for treating and laboratory-based physicians is to understand the contributory role(s) of each of these syndromes, so that the potential value of emerging and innovative pharmacologic strategies can be considered as options in patient blood management.

Knowledge gained regarding the relationship between erythropoietin (EPO), iron, and erythropoiesis has implications for patient blood management, in which the detection, evaluation, and management of patients with anemia is an important component.[1] Iron-restricted erythropoiesis occurs in the presence of one or more iron deficiency syndromes: absolute iron deficiency, functional iron deficiency, and/or iron sequestration. Absolute iron deficiency (absence of storage iron) is the most widely understood and most easily remedied cause of iron-restricted erythropoiesis, occurring commonly in normal individuals. Functional iron deficiency, even in the presence of storage iron and/or oral iron supplementation occurs during intense stimulation by endogenous EPO response to anemia or to pharmacologic therapy with erythropoiesis-stimulating agents (ESAs). In many patient populations, iron sequestration accompanying acute and chronic inflammatory conditions is an underappreciated and common cause of iron-restricted erythropoiesis. Traditional biochemical markers of storage iron such as serum ferritin have limitations in assessment of iron status in patients with anemia of chronic disease. The availability of safer intravenous (IV) iron preparations allows for carefully controlled studies of their value in these patients. Finally, emerging innovative options such as hepcidin antagonists offer promising strategies in management of anemia in a variety of clinical settings. This review will provide an overview of these three iron deficiency syndromes, which can occur separately or in combination in a variety of clinical settings, along with future directions for clinical research.

ABBREVIATIONS: AI = anemia of inflammation; CKD = chronic kidney disease; ESA(s) = erythropoiesis-stimulating agent(s).

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ABSOLUTE IRON DEFICIENCY

Absolute deficiency of storage iron is the most common nutritional deficiency in both underdeveloped and developed countries and is an important public health problem.[2] It is particularly common in young children, pregnancy, and premenopausal women. For developed countries, up to 36% of a general geriatric medical population have been found to be iron deficient.[3] Eleven percent of men and 10.2% of women 65 years and older are anemic, with an overall rate greater than 20% at age 85 and older; of older persons with anemia, one-third are found to be nutritionally deficient, with absolute iron deficiency the most common etiology of these.[4] Finally, aging is increasingly being identified as a proinflammatory state, in which decreased iron absorption in the elderly may be impaired due to an hepcidin-mediated effect. A recent study failed to demonstrate that elderly anemic individuals had elevated hepcidin levels;[5] however, a

TABLE 1. Some conditions associated with absolute iron deficiency*

Dietary
• Growth/development
• Inadequate diet
Women's health
• Pregnancy/breast-feeding
• Menstrual blood losses
Chronic blood loss
• Blood donation
• Nonsteroidal anti-inflammatory drugs (NSAIDs)
• Gastrointestinal neoplasms
• Inflammatory bowel disease
• Peptic ulcer
• Diverticulosis
• Gastrointestinal parasites (developing countries)
Decreased iron absorption
• Celiac disease
• *Helicobacter pylori* infection
• Autoimmune atrophic gastritis

* Modified, from Goodnough.[6]

number of limitations were identified in this retrospective, ad hoc analysis including an absence of gold standard definitions of iron deficiency versus anemia of inflammation (AI), and hepcidin assays being performed on urine rather than serum samples.

Conditions contributing to the development of absolute iron deficiency[6] are listed in Table 1. Blood loss is a major cause of iron deficiency (e.g., females with menses or community blood donors) and is important not only because of its prevalence, but because proper diagnosis and management of the bleeding lesion is important.[7-9] Therapeutic management is primarily focused on repletion of iron stores. Most iron-deficient patients respond well to oral iron therapy, but administration of IV iron may sometimes be required.[10] Developments in parenteral iron therapy have been reviewed recently.[11-13]

FUNCTIONAL IRON DEFICIENCY

Several clinical settings have served as "natural experiments" that have furthered our understanding of the relationship between EPO, iron, and the erythropoietic response to anemia. Finch[14] summarized the knowledge gained primarily from experimental studies in three clinical settings: normal individuals undergoing phlebotomy, patients with hereditary hemolytic anemias, and patients with hemochromatosis. Under conditions of basal erythropoiesis in normal subjects, plasma iron turnover (as an index of marrow erythropoietic response) is little affected, whether transferrin saturation ranges from very low to very high levels. In contrast, the erythropoietic response in individuals with congenital hemolytic anemia, in whom erythropoiesis is increased up to sixfold over basal levels,[15] is affected (and limited) by serum iron levels and by transferrin saturation.[16] Patients with hemochromatosis who underwent serial phlebotomy[17] were observed to mount erythropoietic responses of up to eightfold over basal rates, attributed to the maintenance of very high serum iron and transferrin saturation levels, whereas normal individuals were shown to have difficulty providing sufficient iron to support rates of erythropoiesis greater than three times basal rates.[18] These observations led Finch to identify this state as a "relative iron deficiency," also known as "functional iron deficiency," which he defined as "when increased erythron iron requirements exceed the available supply of iron."[19]

The practice of autologous blood donation in patients scheduled for elective surgery is another clinical setting for studying anemia due to blood loss. The insights gained most recently regarding this relationship between EPO, iron, and erythropoiesis in patients with blood anemia[20] furthered our understanding for effective management of iron-restricted erythropoiesis.[11] Patients undergoing autologous blood phlebotomy may donate a unit (450 $\pm$ 45 mL) of blood as often as twice weekly, until 72 hours before surgery.[21] Oral iron supplements are routinely prescribed. This iatrogenic blood loss is accompanied by a response in endogenous EPO levels that are increased significantly over basal levels, yet remain within the range of normal (4-26 mU/mL).[22] The erythropoietic response that occurs under these conditions is modest.[23] Red blood cell (RBC) production in excess of basal rates is estimated to be 220 to 351 mL (11%-19% RBC expansion,[20] or the equivalent to 1-1.75 blood units), thus defining the efficacy of this blood conservation strategy.

For patients subjected to more aggressive (up to 2 units weekly) phlebotomy, the endogenous EPO response is more substantial. In one clinical trial,[24] a linear-logarithmic relationship was demonstrated between change in hemoglobin (Hb) level and EPO response,[25] also confirmed by experiments in normal subjects.[26] EPO-mediated RBC expansion in this setting is 397 to 568 mL (19%-26% RBC expansion, or the equivalent of 2-3 blood units).[20]

Clinical trials of ESA have also demonstrated a dose-response relationship between EPO and RBC expansion.[27] A study of "very low" dose EPO therapy in autologous blood donors found that 400 U/kg administered over a 2-week interval resulted in clinically significant erythropoiesis.[28] As shown in Table 2,[24,27,29-31] patients treated with ESA therapy during aggressive autologous blood phlebotomy had 358 to 1102 mL (28%-48% RBC) expansion over 25 to 35 days, or the equivalent of 2 to 5 blood units. The range in response (erythropoiesis) to dose (EPO) was not related to patient sex or age,[32,33] suggesting that iron-restricted erythropoiesis accounted for the blunted and variable responses. The importance of functional iron deficiency was underscored by the normal subject with hemochromatosis[31] who had an estimated 79% RBC

TABLE 2. Erythropoiesis during aggressive autologous blood phlebotomy and ESA therapy*

Patients (n/sex)	Total ESA dose (U/kg)	Units donated	Baseline RBC (mL)	RBC (mL) produced	RBC (mL) expansion (%)	Iron therapy
10/female	900	3.4	1285	358	28	IV
24	900	5.2	1949	621	32	PO
10/female	1800	4.3	1293	474	37	IV
26	1800	5.5	2032	644	32	PO
11/female	3600	4.9	1796	701	39	PO
12/male	3600	5.9	2296	1102	48	PO
23	3600	5.4	2049	911	45	PO
18	3600	5.6	2019	856	42	PO
1/male	4200	8	2241	1764	79	Hemachromatosis

* Data are expressed as means. Modified from Goodnough et al.[8]
PO = oral.

volume expansion, far greater even than male patients with normal storage iron who received oral iron supplementation, and who had a maximum 48% RBC volume expansion (Table 2).

The development of functional iron deficiency in healthy subjects treated with an ESA is illustrated in Fig. 1, in which a decrease in transferrin saturation percent was observed within 1 week of initiation of ESA therapy, along with a decline in serum ferritin reflecting transfer of iron from storage pools into synthesis of intracellular Hb.[34] The decrease in transferrin saturation as a marker for development of functional iron deficiency in patients undergoing aggressive autologous blood donation, with or without ESA therapy, is illustrated similarly in Fig. 2.[35] Finally, the enhanced RBC production response to parenteral iron therapy in chronic kidney disease (CKD) patients treated

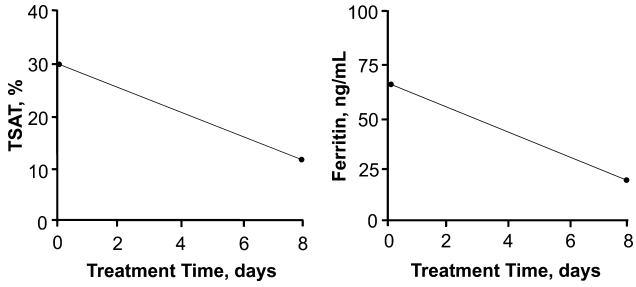

Fig. 1. Change in iron status after initiation of ESA in healthy subjects. Administration of four doses of ESA over 7 days. Administration of ESA use to healthy individuals results in decreased iron saturation, which indicates a decrease in available iron and the development of iron-restricted erythropoiesis. Reproduced from Eschbach et al.,[34] with permission.

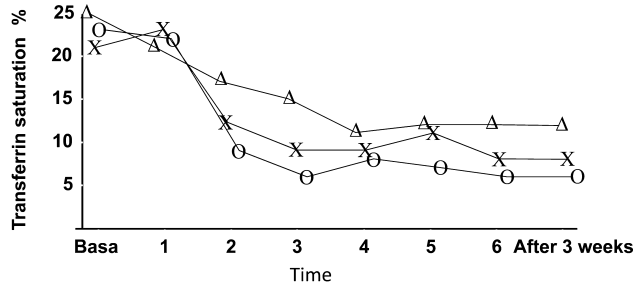

Fig. 2. Impact of erythropoiesis on iron saturation. Mean transferrin saturation in 24 patients receiving placebo (△), 300 U/kg rHu-EPO (χ), or 600 U/kg rHu-EPO (○), supplemented with oral iron. The impact of endogenous EPO-mediated erythropoiesis or ESA-mediated erythropoiesis on iron saturation and ferritin. Patients undergoing autologous blood donation before elective orthopedic surgery are shown at baseline and after treatment with placebo or one of two doses of rHu-EPO at each visit during the donation period. All patients received supplemental oral iron. Reproduced from Mercuriali et al.,[29] with permission from AABB.

with ESA therapy[36] compared to no ESA therapy is shown in Fig. 3.

As was demonstrated more than 50 years ago,[18] the endogenous EPO response stimulates erythropoiesis in patients undergoing autologous blood phlebotomy by up to threefold over basal rate.[27,37] Under conditions of moderate erythropoiesis, serum iron and transferrin saturation for erythron requirements are adequately maintained by storage iron.[37] Little or no benefit to oral iron supplementation was found in two studies,[38,39] whereas a third study[40] found some benefit. IV iron supplementation was not found to be of value in enhancing erythropoiesis under these modest conditions of EPO stimulation.

With ESA therapy, functional iron deficiency occurs even in patients who have storage iron (Figs. 1-3). Despite an eightfold increase in gastrointestinal iron absorption,[41] serum ferritin and transferrin saturation levels decline by 50% with ESA therapy.[42] A fourfold increase in erythropoietic activity is accompanied by declining reticulocyte counts and the appearance of hypochromic RBCs by the second week of ESA therapy.[26,43] In a study of escalating (fourfold) ESA dose administered to patients undergoing aggressive phlebotomy, the marrow erythropoietic index increased from 2.9-fold (with endogenous EPO stimulation) to 3.6-fold over basal rates of erythropoiesis, representing only a 58% increase in erythropoiesis.[20] The superior erythropoietic response in the patient with hemochromatosis[30] indicates that for patients treated with ESA, IV iron therapy is desirable to maintain iron saturation for management of functional iron deficiency. Randomized clinical trials of no iron, oral iron, and IV iron supplementation have confirmed that oncology patients with chemotherapy-induced anemia have superior responses to ESA therapy when treated with IV iron supplementation.[12,44]

IRON SEQUESTRATION

In the past 10 years, understanding of the regulation of iron homeostasis has changed substantially.[45] Hepcidin is a small peptide hormone secreted mainly by hepatocytes and has emerged as the central regulator of iron absorption and plasma iron levels. Hepcidin acts to sequester iron by inhibiting storage iron egress from hepatocytes and macrophages into plasma and also absorption of dietary iron from duodenal enterocytes. Hepcidin exerts its activity by binding and degrading the iron exporter ferroportin,[46] thus reducing the iron available for erythropoiesis (Fig. 4).[47] Molecular defects (e.g., mutations in the gene TMPRSS6) in iron homeostasis can result in inappropriately elevated levels of hepcidin and an iron-refractory iron deficiency anemia because of inhibition of both iron

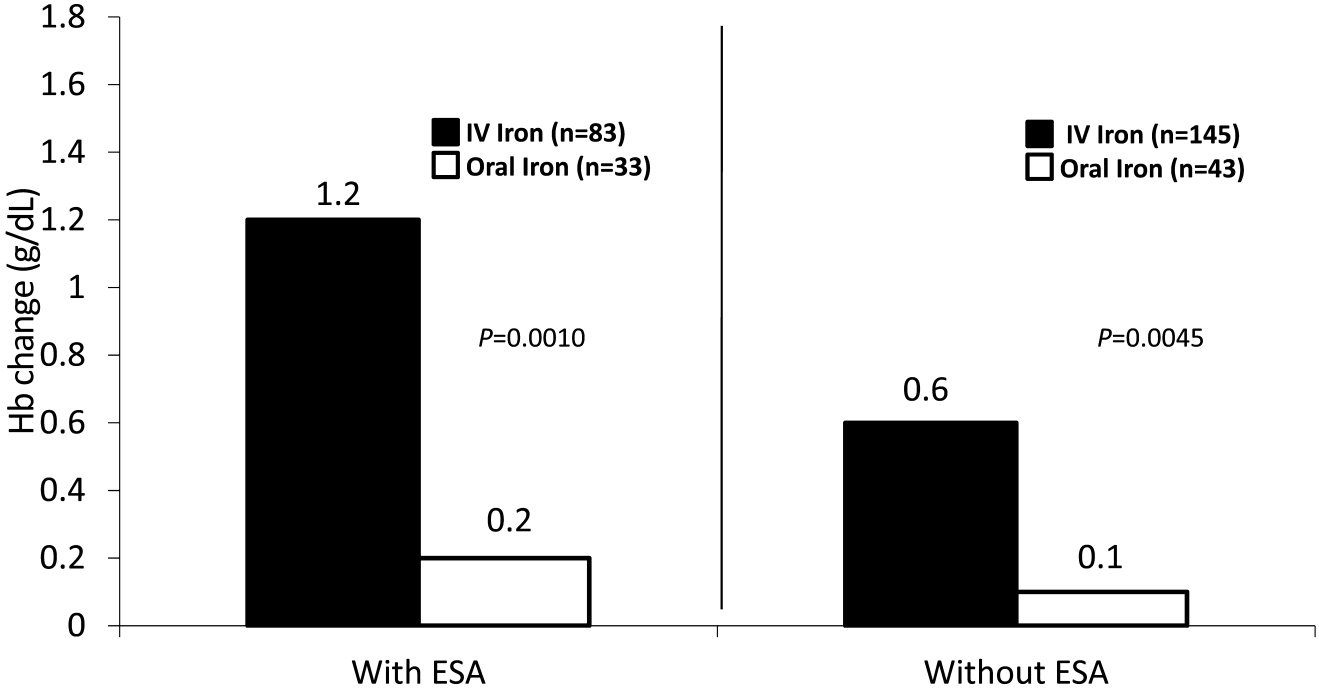

Fig. 3. IV iron supplementation in CKD patients, with and without ESAs. Hb changes on Day 35. Hb changes without ESA therapy are minimal, although better in patients receiving IV iron (ferrymoxytol) compared to oral iron therapy (right panel). In contrast, Hb changes are enhanced substantially with IV iron under intense erythropoiesis stimulation by ESA therapy (left panel). Data derived from Spinowitz et al.,[36] with permission.

absorption from the gastrointestinal tract and iron release from macrophages.[48]

Hepcidin production is regulated by iron and erythropoietic activity.[45] Increased plasma and storage iron stimulate hepcidin production, which in turn inhibits dietary iron absorption. Conversely, iron deficiency and increased erythropoietic activity (e.g., myelodysplastic syndromes or hemolytic anemias) suppress hepcidin to very low levels.[49,50] Low hepcidin allows increased absorption of dietary iron and release of iron storage.[51,52] The supply of additional iron from the diet and stores then permits increased Hb synthesis. A single injection of an ESA in humans significantly decreases serum hepcidin within 24 hours,[50] but EPO does not appear to be a direct regulator of hepcidin.[52] The mechanisms by which erythropoiesis affects hepcidin production are not well understood, but both direct and indirect effects of anemia and erythropoiesis could contribute. Candidate mediators include soluble factors released by erythroid precursors and decreased circulating or stored iron.[53] Hypoxia may alter hepcidin production directly through hypoxia-inducible factor[54] or indirectly via increased EPO production and erythropoiesis.

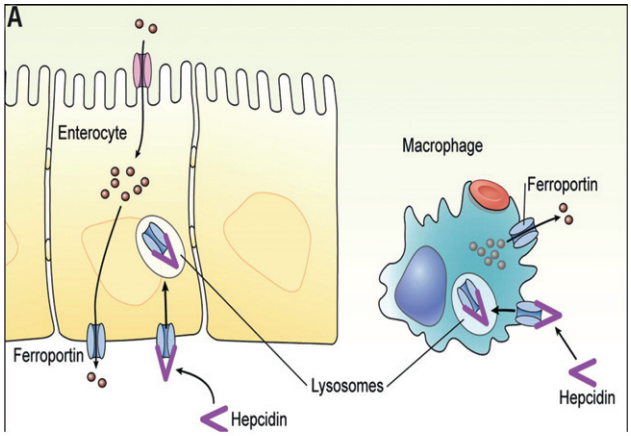

Fig. 4. Hepcidin. Hepcidin causes ferroportin to be internalized and degraded, leading to trapping of iron in enterocytes and reticuloendothelial cells. Reproduced from Andrews,[47] with permission from the American Society of Hematology.

Hepcidin production is strongly increased by inflammation and infection. The increase appears to be mediated by interleukin-6/STAT3, as well as other cytokine pathways.[55] Because of this, hepcidin levels are elevated in a range of inflammatory diseases including rheumatologic diseases, inflammatory bowel disease, infections, critical illness, and malignancies.[51] Increased hepcidin concentrations cause the retention of iron in macrophages and enterocytes, leading to hypoferremia, iron-restricted erythropoiesis, and decreased responsiveness to ESA therapy.[56,57]

ROLE OF HEPCIDIN IN ANEMIA MANAGEMENT

The development of a sensitive, accurate, and reproducible immunoassay for human hepcidin has allowed definition of physiologic and pathologic changes of hepcidin in healthy volunteers and in patients.[49] The assays will be useful in improving our understanding of the pathogenic role of hepcidin in various iron disorders and in the development of appropriate therapeutic interventions. In contrast to changes in ferritin levels, changes in hepcidin

TABLE 3. Potential role of hepcidin in diagnosis and management of anemia

Condition	Expected hepcidin levels	Iron variables	Iron therapy strategies	Potential hepcidin therapy
Absolute iron deficiency anemia (IDA)	Low	Low Tsat and ferritin	PO or IV if poorly tolerated or malabsorbed	No
Functional iron deficiency (ESA therapy, CKD)	Variable, depending on ± CKD	Low Tsat, variable ferritin	IV	Antagonist (if hepcidin levels not low)
Iron sequestration (anemia of inflammation [AI])	High	Low Tsat, normal-to-elevated ferritin	IV	Antagonist
Mixed anemia (AI/IDA or AI/functional iron deficiency)	Variable	Low Tsat, low-to-normal ferritin	IV†	Antagonist (if hepcidin levels not low)
Iron-loading anemias (e.g., ineffective erythropoiesis)	Low	High Tsat and ferritin	Iron chelation therapy	Agonist
Iron-loading anemias treated with transfusion	Normal to high	High Tsat and ferritin	Iron chelation therapy	Agonist

* Updated from Goodnough et al.[11]
† Mixed anemia is a diagnosis of exclusion without a therapeutic trial of iron.
PO = oral; Tsat = transferrin saturation.

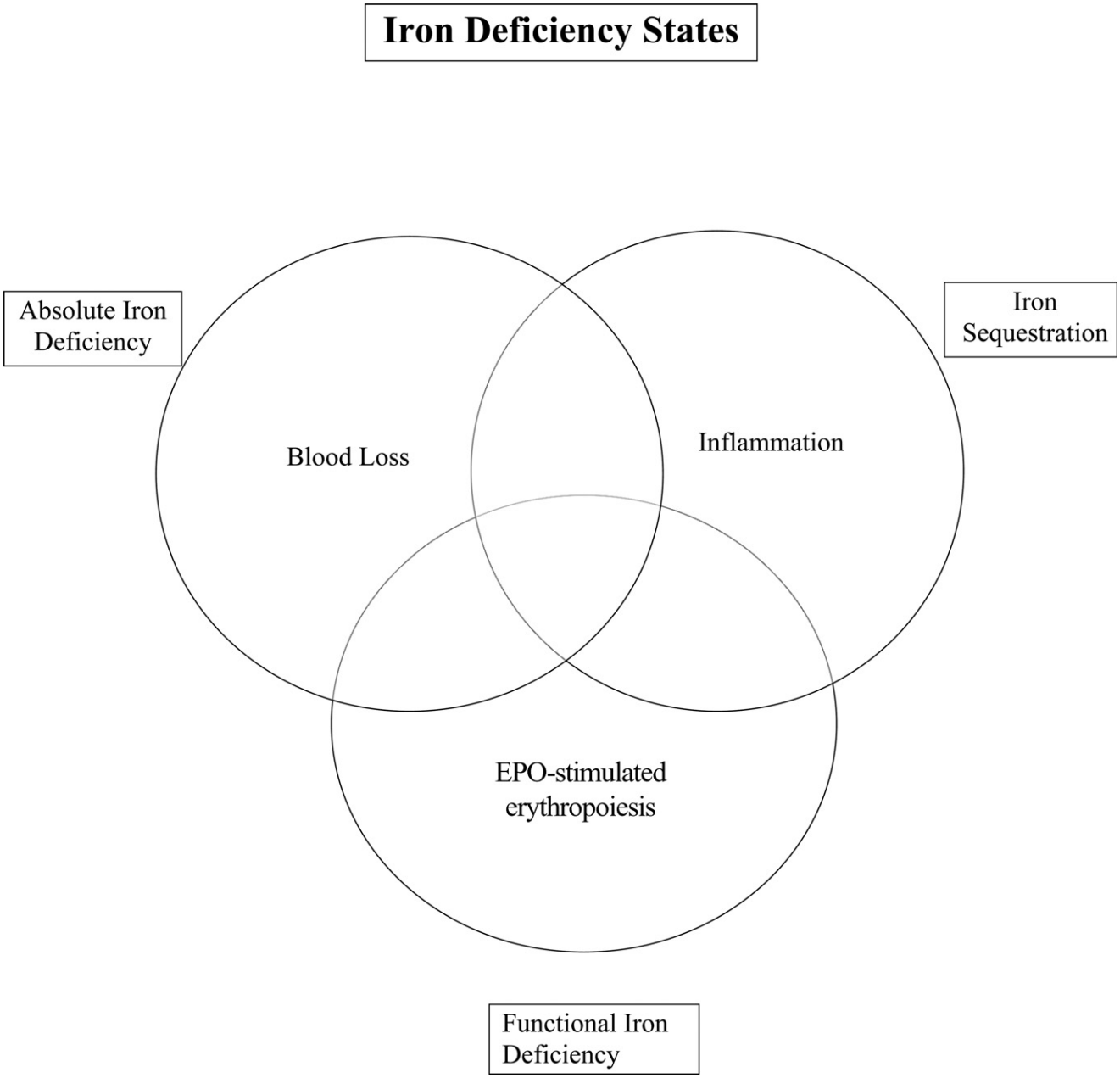

Fig. 5. Iron deficiency syndromes. The relationships between absolute iron deficiency, iron sequestration, and functional iron deficiency are illustrated. Patients can have one or more combinations that all result in iron-restricted erythropoiesis.

concentrations are the cause of, rather than the result of, iron disorders. Hepcidin antagonists may be an effective treatment for patients with inflammatory anemia.[57]

The diagnostic and therapeutic implications for considerations in iron-restricted erythropoiesis depend on the assessment of whether the patient has absolute iron deficiency, an iron sequestration syndrome, and/or functional iron deficiency. It is noteworthy that hepcidin levels are reliably elevated in patients with AI compared to normal values and are low or undetectable in patients with absolute iron deficiency. However, in patients who have mixed problems such as AI coexistent with absolute iron deficiency or functional iron deficiency, hepcidin levels alone may not reliably distinguish among iron deficiency syndromes.[58,59] Expected changes in hepcidin levels and iron variables in various clinical conditions, and in the potential use of hepcidin-targeted and iron therapies in patients with various forms of anemia, are summarized in Table 3.[11,51] As the pathogenic mediator of inflammation, hepcidin would be expected to be high in iron sequestration syndromes. Because hepcidin levels are affected by iron stores,[49] this assay may also identify

patients most likely to respond to iron therapy or identify patients at risk for iron loading.

For patients with mixed presentations, more complex algorithms will need to be developed and tested to provide optimal guidance in the evaluation and management of anemia.[60,61] For example, given that functional iron deficiency can occur in several different conditions, hepcidin levels may be variable: in resistance to ESA therapy due to CKD (because of decreased hepcidin renal clearance), hepcidin levels will be high; in conditions of accelerated erythropoiesis due to endogenous EPO or ESA stimulation, hepcidin levels will be low. It is important to note that several iron deficiency syndromes can coexist in patients: for example, if iron sequestration coexists with functional iron deficiency, hepcidin levels are variable (Table 3). For these patients, hepcidin levels may not help treating physicians arrive at a definitive diagnosis but may give guidance in determining therapy for anemia management (hepcidin antagonists if hepcidin levels are high due to inflammation) or may identify patients who are likely to respond to iron (oral vs. IV) therapy (i.e., hepcidin levels are low with marginal storage iron or accelerated erythropoiesis).

Even under the best of circumstances, oral iron is not well tolerated and patients are often noncompliant.[62] Additionally, hepcidin response in inflammatory conditions inhibits gastrointestinal absorption of oral iron. While evidence in rodents suggests that in the presence of both iron deficiency and inflammation, hepcidin levels are more responsive to the erythropoietic demands for iron than to inflammation and that oral iron can be absorbed,[11] oncology patients respond better to IV iron therapy than oral iron supplementation in patients with chemotherapy-induced anemias treated with ESA.[12,44,63,64] Despite these beneficial effects, IV iron administration may generate oxidative stress and other inflammatory changes. Long-term effects of the IV iron preparations will require careful study in relevant clinical settings.[65,66]

CONCLUSION

Iron-restricted erythropoiesis can arise from one (or more) of three iron deficiency syndromes, depending on underlying pathophysiology. The relationships between these syndromes are illustrated in Fig. 5. An important foundation for patient blood management is successful anemia management, of which iron-restricted erythropoiesis is the most common cause. The challenge for treating and laboratory-based physicians is to understand the contributory role(s) of each of the iron deficiency syndromes that result in iron-restricted erythropoiesis, so that the potential value of emerging and innovative pharmacologic management strategies can be considered. IV iron therapy, emerging hepcidin antagonists, other emerging ESA agents, and the ability to manipulate iron metabolism in patients with inflammatory anemia and a number of other pathologic conditions will each provide important new tools for pharmacologic alternatives in patient blood management.

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CONFLICT OF INTEREST

LTG is a consultant to Amgen, Luitpold, Eli Lilly, and Pharmacosmos.

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