

Hepcidin and Anemia of the Critically Ill Patient

Bench to Bedside

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ANEMIA occurs frequently in critically ill patients, with 60–66% of them having anemia when admitted to the intensive care unit (ICU),¹ and is associated with worse outcomes (such as increased length of stay and increased mortality). Anemia is only partially corrected during ICU stay because recent recommendations have led to a decrease in transfusion triggers. Today the prevalence of anemia in patients being discharged from the ICU is very high, being found in at least 75% of the patients upon final hemoglobin measurement.² This common, and severe anemia at ICU discharge may also be prolonged after the ICU stay. The median time to recovery of anemia is 11 weeks. One observational study reports that more than half of such patients are still anemic at 6 months.³ Although the impact of anemia on rehabilitation after ICU discharge or quality of life has not been fully evaluated, it is well accepted that anemia is associated with worse outcomes for post-operative rehabilitation⁴ and for patients with chronic diseases such as cancer and thus should retain our interest at the bedside and in the research unit. Because blood transfusion is not an option for complete correction of this anemia,¹ one may advocate the use of other treatments, such as erythropoiesis-stimulat-

ing agents (ESA) or iron. ESA efficacy has been studied in critically ill patients and has not proven to be indicated,⁵ at least according to the design of the studies. Iron is needed for erythropoiesis and thus may be indicated for the treatment of this anemia, but iron is also a toxic compound with the ability to induce oxidative stress or promote bacterial growth. A better understanding of iron metabolism regulation may help to define the place of iron in these indications.

The recent discovery of hepcidin (*i.e.*, the master regulator of iron metabolism) has shed new light on the regulation of iron homeostasis and has increased understanding of the physiopathology of anemia in complex clinical situations where several regulatory circuits interfere with iron metabolism, such as anemia of inflammation⁶ and anemia of critically ill patients. This article reviews the link between iron metabolism and anemia in critically ill patients and discusses therapeutic perspectives in this area.

Anemia of Inflammation or Iron-deficiency Anemia?

Anemia in critically ill patients is multifactorial, with the two main contributing factors being inflammation and iron deficiency. These two factors have opposite effects on iron metabolism. Until recently, inflammation was thought to dominate iron deficiency, and the iron profile of critically ill patients was not considered a matter of interest.

Inflammation occurs frequently among critically ill patients, whatever the underlying pathology. The anemia of critically ill patients presents the hallmarks of the anemia of inflammation, with impaired proliferation of erythroid cells, blunted erythropoietin synthesis and response, and most importantly, dysregulation of iron metabolism.⁶ All of these aspects of the anemia of inflammation have been demonstrated in critically ill patients. For example, the plasma of critically ill patients (after trauma or sepsis) has been shown to inhibit the growth of medullar progenitors by inducing their apoptosis. The concentration of erythropoietin in the plasma appears to be lower than expected in anemic critically ill patients^{7,8}; this is the rationale for the use of ESA in ICU patients. Finally, the iron profile of critically ill patients al-

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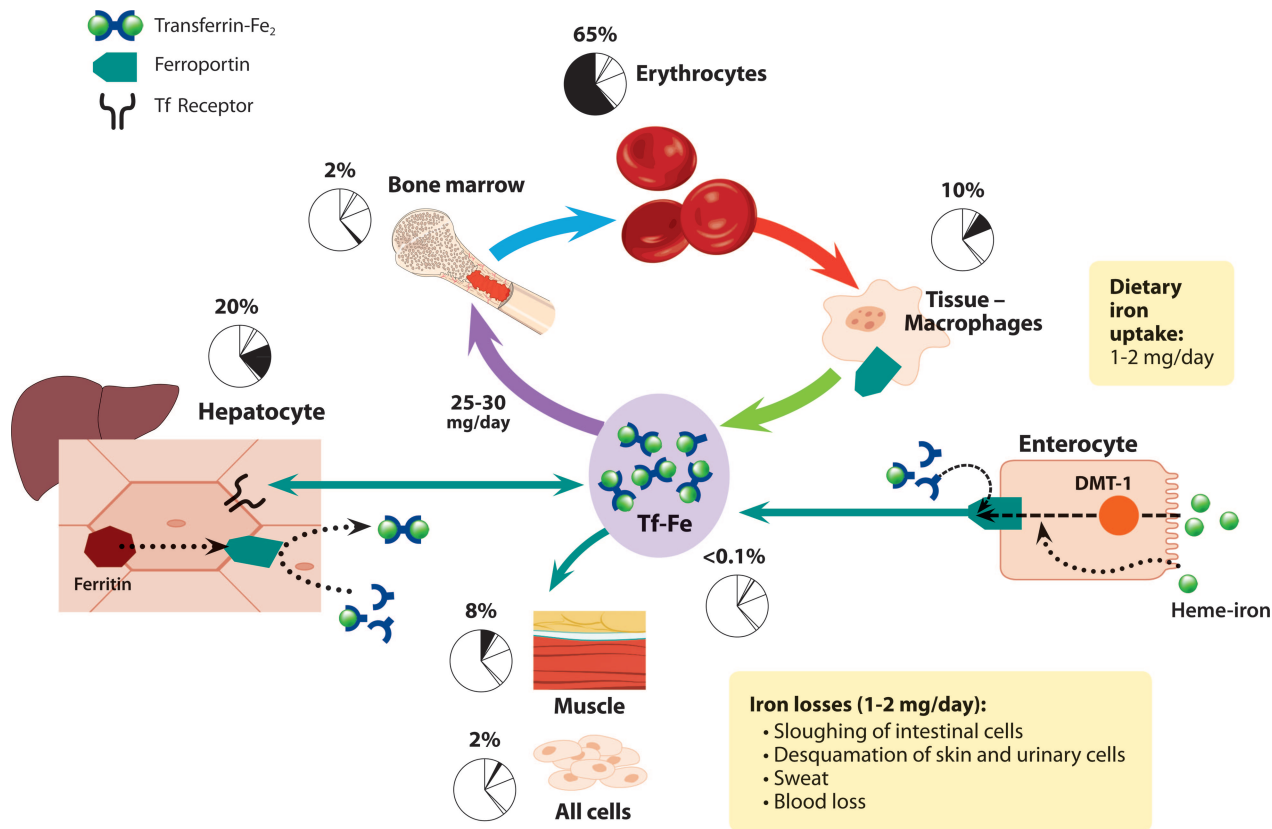


Fig. 1. Distribution of iron in the body. The figure depicts the distribution of the 35–45 mg/kg iron contained in the body. The pie charts represent the relative iron content (percentage) of each compartment. Erythrocytes contain almost two thirds of all body iron. Hepatocytes and tissue macrophages are the main sites of iron storage. Iron turnover is limited, representing less than 1/10³ of the total iron content. Iron is absorbed from the diet in intestinal cells through the apical divalent metal transporter-1 and exported into the blood circulation via the ferroportin. Transferrin transports iron from stores to utilization sites (mainly bone marrow but also other cells, including muscles). DMT-1 = divalent metal transporter-1; Fe = iron; Tf = transferrin.

ways associates low serum iron with high ferritin concentrations, indicative of an inflammatory iron profile.^{7,8} Because ferritin synthesis is induced by inflammation independently of the concentration of iron stores, increased ferritin concentrations are no longer indicative of iron stores in that context of inflammation.⁹ Thus, despite an iron profile suggestive of iron overload (with high ferritin concentrations), these critically ill patients may have iron deficiency.

Critically ill patients do lose large amounts of blood, through blood sampling,¹ surgical bleeding, other invasive procedures (such as drainage, catheters, renal replacement therapies), or occult bleeding. Von Ahsen *et al.*⁸ calculated a median blood loss of 128 ml per day for anemic, critically ill patients, which may represent a median iron loss as high as 64 mg per day. This direct loss of iron could lead to iron deficiency because it is not fully compensated by daily iron intake from the diet, which is small (approximately 1–2 mg in normal conditions), and is further decreased by inflammation because of the hepcidin-mediated repression of ferroportin (see section Hepcidin: The Master Iron Metabolism Regulator and fig. 1). Iron deficiency may affect as many as 30–40% of critically ill pa-

tients.^{9–11} We describe the diagnostic tools for iron deficiency after discussing iron metabolism and its regulation.

Iron Metabolism Overview

Iron is a paradoxical element; it is essential for living organisms and potentially toxic. Indeed, iron has the ability to accept and donate electrons readily, interconverting between the readily soluble ferrous form (Fe²⁺) and the insoluble ferric form (Fe³⁺). This capacity makes iron essential for living because it is implicated in oxygen transport and in other reactions of electron transfer, nitrogen fixation, or DNA synthesis. It can also be toxic because of its capacity to react with oxygen and to catalyze the production of free radicals. This duality is found in human pathology: iron deficiency (because of poor iron intake in the diet, abnormal blood losses, and others) presents with anemia and fatigue, whereas iron overload (mainly in hereditary hemochromatosis and after repeated blood transfusions) induces multiple organ dysfunction (including liver fibrosis, cirrhosis, cardiomyopathy, and diabetes). This explains why iron homeostasis must be finely tuned to avoid deficiency and excess.

Iron distribution in the organism is a nearly closed circuit (fig. 1). Adult humans normally have 35–45 mg iron per

kilogram body weight (approximately 3–4 g in total), and the global iron turnover through losses (because of bleeding or cell desquamation) and dietary uptake (by the duodenal cells) is only approximately 1–2 mg per day. Thus, the organism mainly depends on iron recycled by macrophages from the heme moiety after erythrophagocytosis (*i.e.*, the recycling of senescent erythrocytes). Indeed, more than two thirds of the body's iron content is incorporated into hemoglobin, either in bone marrow erythroid progenitors or in circulating erythrocytes. During aging, the erythrocyte membrane undergoes biochemical modifications (with phosphatidylserine externalization, lipoprotein peroxidation, and formation of neoantigens). These senescent erythrocytes are recognized and phagocytosed by macrophages of the reticuloendothelial systems. After heme catabolism, iron is released in the macrophage cytosol and then recycled toward the circulation or stored in ferritin molecules. In the plasma, transferrin binds the newly released iron to allow its transportation from storage sites (mainly tissue macrophages, including the spleen and to a lesser extent the liver) to utilization sites (mainly the bone marrow and to a lesser extent the myoglobin-containing muscles). The daily bone marrow erythropoiesis requires 25–30 mg iron, which is mainly provided by erythrophagocytosis. However, the amount of iron present in the plasma at any time is very small (approximately 3 mg) compared with the total amount of iron in the body and the iron needed daily for erythropoiesis, which leads to the necessity of fine-tuning the plasma iron turnover.

Many years ago it was suggested that intestinal iron absorption was under the tight control of two major “regulators,” a store regulator and an erythroid regulator.¹² The first one was aimed at increasing iron uptake in the event of iron deficiency, and the second one was aimed at increasing iron uptake in the event of stimulation of erythropoiesis. The discovery of hepcidin in the early 2000s helped to put a name on these two regulators, which consist in a single peptide: hepcidin.¹³ This discovery ignited an explosion of research in the field of iron biology, leading to a better understanding of iron homeostasis.¹⁴

Hepcidin: The Master Iron Metabolism Regulator

Hepcidin is a small, 25-amino acid peptide produced mainly by the liver. Some other tissues or cells, including kidney, macrophages, adipocytes, and pancreatic β cells, have been shown to synthesize hepcidin. Hepcidin is produced as a prepropeptide of 84 amino acids. The prohepcidin has been shown to be biologically inactive. The process of hepcidin maturation and secretion is incompletely understood but probably requires cleavage by furin enzymes.

Hepcidin acts by binding to ferroportin, the sole known iron exporter, causing its internalization and degradation in the cytosol. This degradation of ferroportin prevents the release of intracellular iron content. Ferroportin is expressed mainly in macrophages and duodenal cells, allowing, respectively, iron recycling and its digestive absorption. Hepcidin may thus inhibit the release of iron from tissue macrophages,

leading to iron-restricted erythropoiesis, or from the duodenal cells after its uptake from the digestive lumen, leading to dietary iron deficiency. Thus, hepcidin acts as a “hyposideremic” hormone, aiming at inhibiting iron absorption and reducing the concentration of iron in the blood.

Hepcidin synthesis is finely regulated (fig. 2). Iron overload and inflammation induce hepcidin synthesis, whereas iron deficiency, hypoxia, and erythroid expansion repress hepcidin synthesis. The molecular mechanisms implicated in these complex regulations are not fully understood (see Hentze *et al.*¹⁴ for review), but the induction of hepcidin synthesis by inflammation has been proven to be interleukin-6-dependent. The infusion of interleukin-6 in healthy human volunteers induces a rapid (within hours) urinary excretion of hepcidin, reflecting increased hepcidin synthesis and a concomitant decrease in plasma iron. The induction of hepcidin by inflammation is thought to contribute to the iron-restricted erythropoiesis observed during chronic diseases, ultimately leading to the “anemia of chronic disease” (or anemia of inflammation).⁶ However, iron deficiency and stimulation of erythropoiesis can inhibit hepcidin synthesis. This response appears to be multifactorial, depending on the model considered. Matriptase-2, a membrane-bound serine protease expressed in hepatocytes, seems to play a role in repressing hepcidin synthesis in conditions of iron deficiency.¹⁵ In animal models, hypoxia or injections of erythropoietin have been shown to repress hepcidin synthesis through hypoxia-inducible factor or CCAAT/enhancer-binding protein- α pathways, respectively.¹⁴ However, in human pathology, very few observations have been made to explain the repression of hepcidin. Tanno *et al.*¹⁶ found very high concentrations of growth differentiation factor 15, a member of the transforming growth factor β family produced by late erythroblasts, in patients with β -thalassemia syndromes and showed that this factor was able to repress hepcidin synthesis in hepatocytes. However, these data have never been confirmed. As stated, both stimuli (*i.e.*, either inflammation or iron deficiency or erythropoiesis stimulation) may coexist in the context of critically ill anemia and may interact differently with hepcidin synthesis.

Diagnosis of Iron Deficiency During Inflammation: A Place for Hepcidin?

Until recently, the induction of hepcidin synthesis by inflammation was considered to be the rule. However, using animal models, we and others demonstrated that hepcidin can be repressed despite inflammation^{17–19} and that this repression is associated with mobilization of splenic iron.¹⁸ These observations reinforce the concept of iron-deficiency anemia coexisting with anemia of inflammation.⁶ Thus, hepcidin concentration may be helpful for the diagnosis of iron deficiency in the context of inflammation.

Iron-deficiency diagnosis in the context of inflammation is difficult because the usual indicators of iron deficiency are no longer usable.⁹ In the presence of inflammation, serum

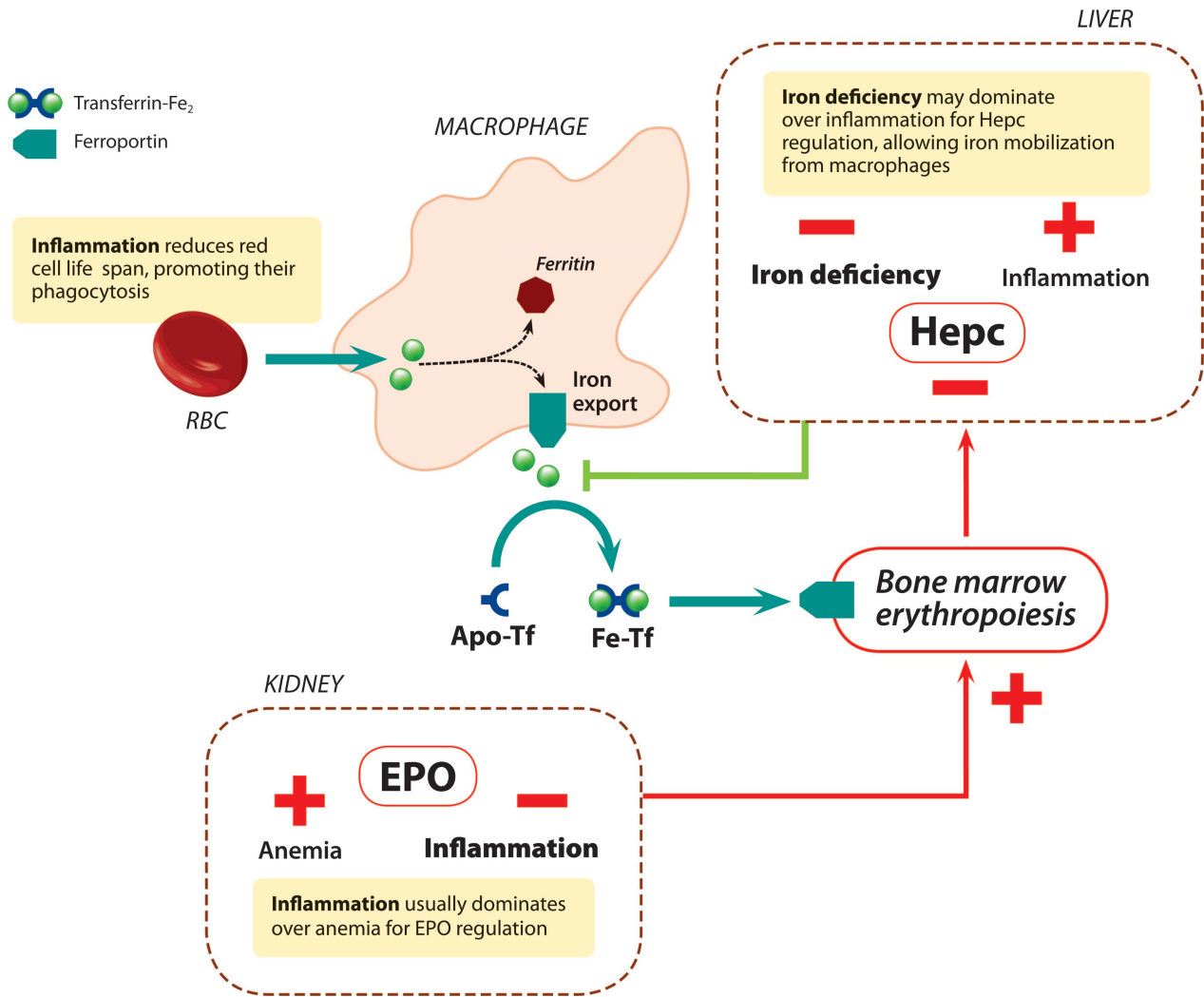


Fig. 2. Regulation of iron metabolism in the anemia of a critically ill patient. Two opposite stimuli regulate hepcidin, the master regulator of iron metabolism. Inflammation induces hepcidin synthesis, whereas iron deficiency, blood loss, and erythropoiesis stimulation (modulated by erythropoietin synthesis) repress it. A low hepcidin concentration is required to allow iron export and its utilization for erythropoiesis. Apo-Tf = apo-transferrin (i.e., transferrin free of iron); EPO = erythropoietin; Fe-Tf = transferrin binding iron; Hepc = hepcidin; RBC = erythrocytes.

iron and transferrin saturation are decreased (as occurs in iron deficiency), whereas plasma ferritin is increased in the absence of any change in total body iron. Thus, new biologic markers have been proposed for the diagnosis of this combined occurrence of iron deficiency and anemia of inflammation (see table 1).

Blood analyzers can be used to detect hypochromic erythrocytes, which are the result of an iron-restricted erythropoiesis. Schematically, one may analyze all circulating erythrocytes, and a value of more than 10% hypochromic erythrocytes (normal, less than 2.5%) is indicative of an iron-restricted erythropoiesis during the past 3 months (the lifespan of erythrocytes). One may also measure the reticulocyte hemoglobin content, with a value of less than 28 pg considered indicative of an iron-restricted erythropoiesis during the past 2–3 days (the lifespan of reticulocytes). A low reticulocyte hemoglobin concentration on admission recently was shown to be associated with a higher

transfusion rate.¹⁰ Of note, each manufacturer develops its own parameters, using the same principle but different measures and different cutoff values.

Erythrocyte zinc protoporphyrin is also proposed as an indicator of iron-restricted erythropoiesis. During normal erythropoiesis, iron is incorporated into protoporphyrin IX to form heme. If little iron is available in the bone marrow, zinc is substituted for iron, leading to the formation of zinc protoporphyrin. Thus, increased erythrocyte zinc protoporphyrin is indicative of iron deficiency. Furthermore, the value of erythrocyte zinc protoporphyrin is supposed to be unaffected by inflammation.

Determination of the soluble transferrin receptor is widely proposed for the diagnosis of iron deficiency and of the combined situation of iron deficiency and inflammation. Transferrin receptors are expressed mostly at the cell surface of erythroid progenitors and implicated in internalization of

Table 1. Biological Parameters of Iron Metabolism

	Normal	Iron Deficiency	Anemia of Inflammation	Iron Deficiency and Inflammation
Bone marrow iron	2-3	0-1	2	1-2
Iron	0.7-1.8 mg/l 12-35 μ mol/l	↓↓	↓↓	↓↓
Transferrin	1.8-2.85 g/l 25-25 μ mol/l	↑↑	↓↓	N or ↓
Tf saturation	20-50%	↓↓ <16%	↓↓ <20	↓↓ <20
Ferritin	30-300 μ g/l 50-670 pmol/l	↓↓ <30 μ g/l (women <12 μ g/l)	↑↑ >100 μ g/l	↑ Variable 100 to 300 μ g/l
Hypochromic RBC	1-5%	↑↑	↑	↑↑
CHr	28-35 pg	↓↓	N or ↓	↓
Erythrocyte zinc protoporphyrin	30-80 μ mol/mol heme*	>200	>100	>200
sTfR	0.83-1.76 mg/l 1.9 - 4.4 mg/l †	↑↑	↓↓	↑
sTfR/log ferritin	<0.7 (1.5) or <2 (4) †	↑↑ >5 or 4	↓ <0.7 or 2	↑ >0.7 or 2
Hepcidin	No standard ref.	↓↓	↑↑	N or ↓
C-reactive protein	<10 mg/l*	N	↑↑	↑

*Values vary according to the different assays. †For respectively the Siemens Healthcare Diagnostics (Deerfield, IL) (formerly commercialized by Dade Behring SA) and the Roche diagnostics (Meylan, France) sTfR assays.

CHr = reticulocyte hemoglobin content; N = value in the normal range; RBC = red blood cells; sTfR = serum transferrin receptor; Tf = transferrin.

holotransferrin. Thus, they are “the gate of iron” for erythropoiesis. Transferrin receptor synthesis is determined by bone marrow erythropoietic activity and strongly increased when iron supply to erythroid cells is insufficient. The soluble transferrin receptor is a truncated form of the cell surface transferrin receptor, and its concentration in serum increases in iron-deficiency anemia or after hemolysis or ineffective erythropoiesis. The ratio of soluble transferrin receptor to the ferritin log (called the ferritin index) is proposed as a marker to differentiate between anemia of inflammation and the combined occurrence of iron deficiency and anemia of inflammation. There is no gold standard for soluble transferrin receptor measurement, so the cutoff values vary from one assay to the other.

All of these variables have to be combined to diagnose iron deficiency in the presence of inflammation. Finally, complex algorithms have been proposed,^{6,9} but they are not clinically validated, and the cutoff values for each variable are not known. The choice of a cutoff value is important, although a more appropriate option could be to propose two

cutoff values separated by a “gray zone”: the first one chosen to exclude the diagnosis with near certainty, and the second to include the diagnosis with near certainty.²⁰

Because hepcidin is central to iron metabolism and its synthesis is influenced by inflammation and iron deficiency in opposite ways, it may be a marker of iron deficiency, even in the presence of inflammation. In addition, many hepcidin assays have been developed recently.²¹ Hepcidin has been shown to be decreased in patients with iron deficiency and anemia of inflammation to virtually undetectable concentrations¹⁹ or to normal values.²² We found that the hepcidin concentration is an accurate diagnostic tool for determining iron deficiency in critically ill anemic patients¹¹ and may thus represent an alternative to these complex algorithms. We chose a cutoff value for iron deficiency as a hepcidin concentration of less than 130 ng/l, with a specificity of 0.85.¹¹ However, until now the efficacy of iron treatment based on hepcidin concentration has not been demonstrated. This better understanding of iron metabolism may promote new therapeutic propositions, including iron treatment.

What Are the Implications for the Treatment of Anemia in Critically Ill Patients?

The main treatment of anemia in critically ill patients is blood transfusion. However, recommended transfusion triggers tend to decrease (because transfusion may be harmful, inducing immunomodulation; the risk of transfusion-related acute lung injury; and others), and blood resources are becoming sparse.

For the past 20 years, ESA have been given to critically ill patients to prevent the development of severe anemia and reduce blood transfusions. However, a recent meta-analysis of nine randomized control trials demonstrated only a small reduction in the number of patients undergoing transfusion (odds ratio, 0.73; 95% confidence interval, 0.64–0.84; $I^2 = 54.7\%$) and a small reduction in the number of units transfused per patient (odds ratio, -0.41 ; 95% confidence interval, 0.10–0.74; $I^2 = 79.2\%$).⁵ To date there is no recommendation for the use of ESA in critically ill patients. However, it has to be underscored that only one study administered intravenous iron in association with ESA. In anemia of chronic kidney disease, it is well recognized that response to ESA is greatly enhanced by intravenous iron. In addition, in the study in which intravenous iron was associated with ESA treatment, the effect of erythropoietin treatment was greater in terms of reduction in blood transfusion and increase in hemoglobin concentrations.²³ There may still be room for ESA treatment in critically ill patients (not as a systematic treatment for all critically ill patients, but as a treatment for those expected to have longer ICU stays) given in association with iron to increase the hemoglobin concentration at discharge. Indeed, some patients have prolonged anemia after ICU discharge, with persistent low erythropoietin concentrations.³

But iron may also be proposed to correct iron deficiency, even in the presence of inflammation, as it is now proposed to treat patients with anemia associated with cancer.^{6,24} Iron may be given using the enteral or the intravenous routes. Ferrous iron is used for the enteral route and requires a mildly acidic medium (*i.e.*, no concomitant use of proton pump inhibitors) and ascorbic acid. However, its absorption may be reduced (secondary to the hepcidin-linked decrease in ferroportin in duodenal cells) or because of the frequently occurring gastrointestinal side effects. Intravenous iron allows giving much higher doses with few side effects and no difficulty of absorption. However, the only available study of intravenous iron⁷ shows no beneficial effect on erythropoiesis when used without ESA, but it had a limited statistical power, and iron was not given according to a diagnosis of iron deficiency. The only study of treatment of iron deficiency in critically ill patients is that of Pieracci *et al.*, which explores enteral iron supplementation (ferrous sulfate 325 mg three times daily).²⁵ In the study, oral iron was associated with a reduction in the transfusion rate in patients with baseline iron deficiency and no increased risk of infection. Iron may thus be proposed to correct iron deficiency and/or en-

hance response to ESA in critically ill patients, but additional studies are needed to rule out the potential risks of iron treatment (*i.e.*, oxidative stress induction, increased risk of infection). Indeed, the use of large amounts of iron, exceeding the transferrin iron-binding capacity, may promote free iron, which is known to induce oxidant stress through the Fenton reaction. There is also a link between iron and infection, supported essentially by experimental data on microorganisms and retrospective studies in hemodialysis patients showing an association between hyperferritinemia and the likelihood of infection. But the available observational studies in postoperative or critically ill patients show no association between intravenous iron and infection risk.

We think iron should be given to critically ill patients only for iron deficiency, which is best defined by a low hepcidin concentration. The total amount of iron needed may be calculated according to the degree of anemia and to the target concentration of hemoglobin using the formula: iron deficit = body weight (kg) \times (target hemoglobin – actual hemoglobin) \times 2.4. Iron should be given using fractionated injections to avoid exceeding the iron-binding capacity of transferrin and to limit induction of hepcidin synthesis. Additional clinical studies are needed to validate these proposals.

Finally, because hepcidin is responsible for iron-restricted erythropoiesis, the use of hepcidin antagonists (either direct, through neutralizing antibodies, or indirect, through inhibitors of the stimulatory pathways for hepcidin transcription) may be advocated. There are some initial encouraging experimental data,¹⁴ but they are too preliminary to be proposed for clinical practice.

In conclusion, better knowledge of iron metabolism, including the discovery of hepcidin, may allow for easier recognition of iron deficiency in the presence of inflammation in critically ill patients. This opens new areas for research in exploring the role of iron treatment for these patients.

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