Anemia of Chronic Disease and Iron Deficiency Anemia in Inflammatory Bowel Diseases: Pathophysiology, Diagnosis, and Treatment

Natalia Murawska, Adam Fabisiak,* and Jakub Fichna, PhD, DSc*,†*

Abstract: Anemia coexists with inflammatory bowel disease (IBD) in up to two-thirds of patients, significantly impairing quality of life. The most common types of anemia in patients with IBD are iron deficiency anemia and anemia of chronic disease, which often overlap. In most cases, available laboratory tests allow successful diagnosis of iron deficiency, where difficulties appear, recently established indices such as soluble transferrin–ferritin ratio or percentage of hypochromic red cells are used. In this review, we discuss the management of the most common types of anemia in respect of the latest available data. Thus, we provide the mechanisms underlying pathophysiology of these entities; furthermore, we discuss the role of hepcidin in developing anemia in IBD. Next, we present the treatment options for each type of anemia and highlight the importance of individual choice of action. We also focus on newly developed intravenous iron preparations and novel, promising drug candidates targeting hepcidin. Concurrently, we talk about difficulties in differentiating between the true and functional iron deficiency, and discuss tools facilitating the process. Finally, we emphasize the importance of proper diagnosis and treatment of anemia in IBD. We conclude that management of anemia in patients with IBD is tricky, and appropriate screening of patients regarding anemia is substantial.

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Key Words: anemia, inflammatory bowel diseases, hepcidin, iron deficiency anemia, anemia of chronic disease

The group of inflammatory bowel disease (IBD), which consists mainly of Crohn's disease (CD) and ulcerative colitis, is a group of gastrointestinal tract disorders with unknown etiology. Patients with IBD usually had diarrhea, abdominal pain, nausea, weight loss, chronic fatigue, or gastrointestinal bleeding. Of note, approximately one-third of IBD patients present at least 1 extraintestinal feature,[1–3] such as arthropathies, and also hepatobiliary, ocular, or cutaneous diseases. Anemia, the leading hematological symptom, could be perceived as a manifestation or complication of IBD. It has been stated that anemia is too common among patients to be regarded as a complication and is consistently linked with IBD.[4] Nevertheless, the prevalence of anemia in IBD ranges from 4% to 67%.[5] Gerasimidis et al[6] found even a greater number among children with IBD; up to 72% of pediatric patients could be anemic at diagnosis.

Anemia in IBD is multifactorial and requires special approach from physician, as it further reduces an already impaired quality of life (QOL)[7]; however, a successful management of anemia was shown to improve QOL.[8] The most frequent in IBD is iron deficiency anemia (IDA),[9] and the main factors contributing are either chronic intestinal bleeding or poor absorption (i.e., diet low in heme iron, undergone restorative proctocolectomy procedure, or CD itself).[10] Although B12 vitamin and/or folate deficiencies are believed to be of minor importance in anemic patients, Bermejo et al[11] showed significant proportions of patients in which they occur.

Leaving aside the micronutrient deficiencies, the primary cause of anemia in patients with IBD is anemia of chronic diseases (ACD). Pathophysiology of ACD is closely associated with hepcidin, the protein hormone produced mainly by the liver (for review see Refs. 12,13).

IRON DEFICIENCY ANEMIA

IDA is the most common anemia occurring in IBD, and its prevalence ranges from 36% to 76% patients.[14] Noteworthy, a study conducted by Vijverman et al showed that the prevalence of mild-to-moderate anemia between 1993 and 2003 has decreased, what may results from a wider availability of immunosuppressive treatment. However, the prevalence of severe anemia remained unchanged.[15]

The turnover of iron is shown in Figure 1. Healthy people supply in a diet approximately 5 to 15 mg of elemental iron and 1

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From the *Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, Lodz, Poland; and †Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland.

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Total body iron deficit (TID) sites are contained in the Appendix 1.

Core tip: The goal of this review is to discuss therapeutic options for anemia in IBD and point out difficulties in the course of anemia treatment. Also, perspectives on the hepcidin-dependent pathways as a novel pharmacological target are presented.

Reprints: Jakub Fichna, PhD, Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, Mazowiecka 6/8, 92-215 Lodz, Poland (e-mail: jakub.fichna@umed.lodz.pl).

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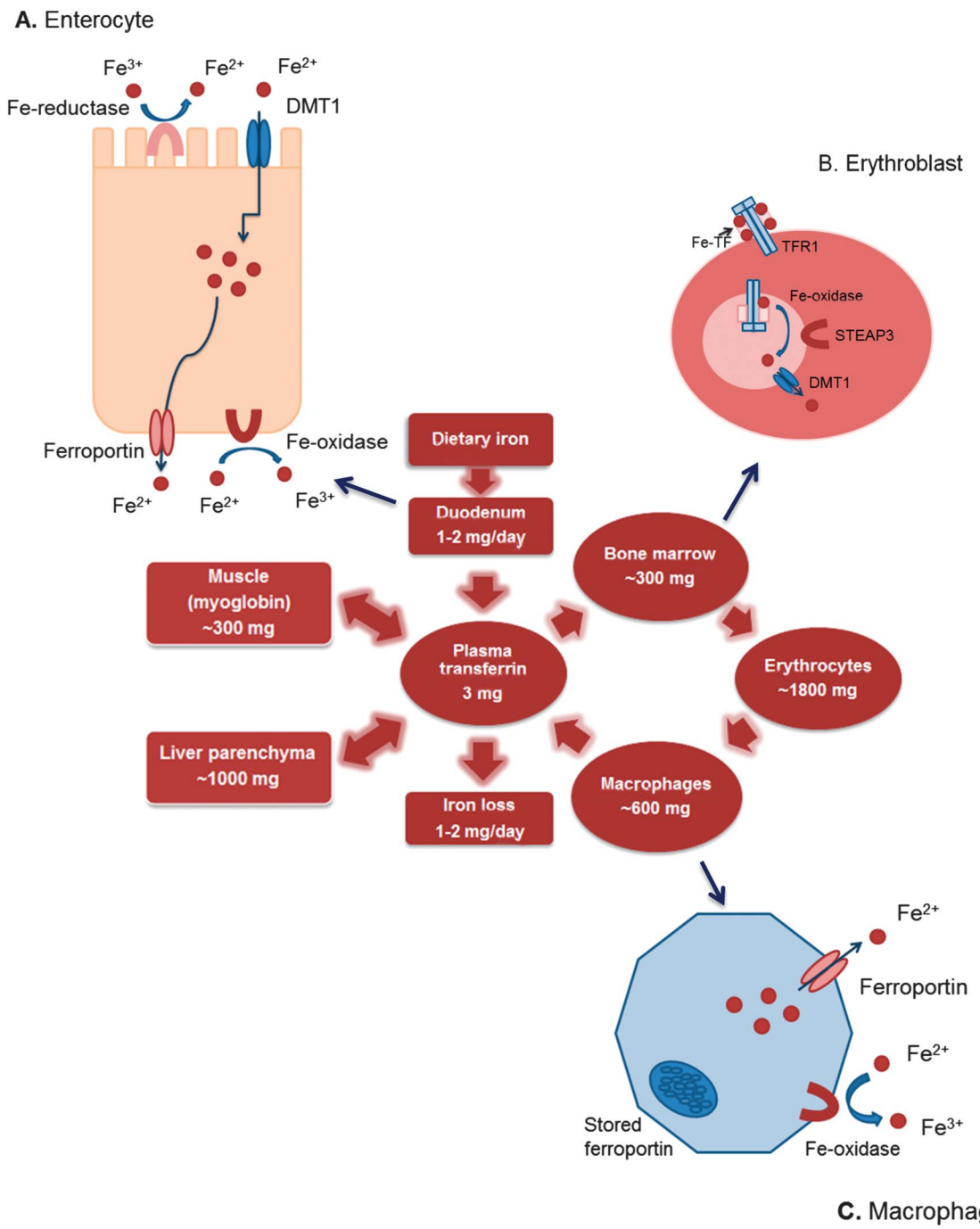

FIGURE 1. Iron turnover and homeostasis in the human body. Scheme A depicts transport of nonheme iron across the intestinal enterocytes. Around 1 to 2 mg of iron is absorbed by the duodenal mucosa per day. Iron bonded with transferrin in plasma comprises approximately 3 mg in total; erythroid precursors in bone marrow and erythrocytes contain most of the iron. Iron absorption in erythroblasts proceeds through transferrin cycle (Scheme B). Senescent red blood cells are used in reticuloendothelial macrophages, which supply iron for synthesis of new erythrocytes. Scheme C shows iron recycling in tissue macrophages. In the liver around, 1000 mg of iron is stored in the form of ferritin and hemosiderin, and 300 mg is found as myoglobin in muscles. Around 1 to 2 mg of iron is lost each day in balance of physiological iron metabolism because of epithelial cell exfoliation. Data from Refs. 16,17 modified. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

to 5 mg of heme iron daily, of which approximately 1 to 2 mg is absorbed in the intestine, mainly in the duodenum; only some amount of iron is lost with feces.[18] Heme iron, which is supplemented mainly with animal products, for example, myoglobin meat, poultry, and fish, is easily absorbable, and its absorption is not influenced by other dietary components. Conversely, nonheme iron can be found mostly in plant food. Absorption of inorganic iron is low and depends on many dietary constituents. Phytate, oxalate, polyphenols, and tannin, frequently occurring in plants, diminish the uptake of inorganic iron.[19,20] Some drugs, e.g., commonly used proton pump inhibitors also weaken the process. Finally, avoidance of ingesting dairy products could facilitate iron supplementation, as calcium inhibits absorption of both iron forms.[21]

Oral Iron Therapy in IBD

The impairment of iron assimilation occurs in some gastrointestinal tract diseases, e.g., *Helicobacter pylori* infection or celiac disease.[22] Main reasons for IDA in patients with IBD are chronic blood loss caused by mucosal damage (ulcerations), and also impairment of iron absorption in inflamed mucosa of duodenum and upper jejunum in patients with CD.[23,24] Of note, recently published studies showed that the iron uptake is normal in patients with inactive or mild IBD.[25,26] Moreover, patients with severe IBD also avoid food because of exacerbated abdominal symptoms decreasing diverse trace element intake.[27] Eventually, ineffective erythropoiesis and inadequate response of erythropoietin to available iron may also result in iron deficiency.[28]

Current treatment of IDA is based on various oral iron supplements. Ferrous fumarate, ferrous sulfate, and ferrous gluconate, which contain ferrous form of iron (33%, 20%, and 12% of elemental iron, respectively), are the most frequently used oral iron preparations. Oral iron is popular among physicians because of the lower cost, a noninvasive, easy method of administration and established safety profile. Because of the increase in iron bioavailability in acidic environment, combination with vitamin C is used to optimize the treatment. The dose of elemental iron recommended by The Centers for Disease Control and Prevention is 30 mg/day for prophylaxis of IDA in IBD and 50 to 60 mg/day for treatment.[29] The earliest response to the treatment is observed after 4 days of the first dosage when the level of reticulocytes in blood starts to increase (with maximum level at 7–10 day after beginning of the treatment). Concurrently, the level of hemoglobin elevates by 1–2 g/dL every 2 weeks.[30,31] Of note, an appropriate level of iron is not achievable briefly; recommended therapy should last for up to 5 months or for at least 3 months after fully replenishing iron stores.[14,32] It also needs to be underlined that oral iron supplementation is used in mild-to-moderate IDA (Hb $\geq$10 g/dL, ferritin $<$100 ng/mL, transferrin saturation $<$20%, and total iron deficit: 1300–1800 mg).[33]

Although oral iron is a very common option for anemia and iron deficiency in patients with IBD, several studies suggest that it is not the most appropriate form in the basic treatment. Lugg et al[34] reported that 2 of 3 patients with IBD had gone through ineffective oral iron therapy and side effects had accompanied in 51% cases. Most patients are dissatisfied with their oral iron treatment mainly because of tolerability issues. Oral iron supplements may cause a number of adverse effects, especially in the digestive system, such as epigastric pain, nausea, flatulence, and diarrhea in up to 20% of patients.[33] Consequently, side effects can lead to disruption of treatment in up to 21% of patients with IBD.[5] Moreover, orally administered iron is absorbed only partially, and nonabsorbed iron salts can be toxic for intestinal mucous membrane, probably through the Fenton reaction.[35] High doses can also aggravate inflammation because nonabsorbed salts seem to display proinflammatory action.[36] Finally, one of the latest studies demonstrated that response to oral iron therapy depends on the level of C-reactive protein (CRP) in patients with IBD, and high baseline CRP is linked with a weaker hemoglobin response in those patients.[37] Therefore, iron should be administered orally to patients with IBD only with inactive or mild disease.

To circumvent the above-mentioned problem, novel oral iron preparations are being designed. For example, ferric maltol, a combination of iron and maltol (3-hydroxy-2-methyl-4-pyrone), is currently in phase 3 clinical trial program, and the results are encouraging (NCT01340872, NCT01352221). Ferric maltol exhibits a satisfactory safety profile and rapidly normalizes Hb levels, what makes it an ideal drug to be used to treat mild-to-moderate IDA in adult patients with IBD, who are intolerant to currently available oral iron preparations, preceding the implementation of parenteral iron.[38]

Intravenous Iron Therapy in IBD

Although more than two-thirds of patients with IBD are still receiving oral iron supplementation,[39] most of the patients are content with intravenous (IV) therapy,[40] which seems a good alternative. For the first time, IV iron was used successfully to treat IDA in chronic disease in the first half of the twentieth century.[41] However, this approach became more popular when new preparations were designed[42–45] because of their higher efficiency, safety, and reduced occurrence of anaphylactic type reaction.[46]

In general, IV iron therapy is tolerated better by patients who had to interrupt oral iron supplementation because of burdensome adverse effect; the IV iron should, therefore, be used when intolerance to oral preparations occurs. Also, patients with insufficient response (Hb increase below 2 g/dL within 4 weeks) should be considered to receive IV iron therapy instead of oral supplements.[47] Moreover, IV iron seems more effective in improving hemoglobin level and has been proven more effective in increasing ferritin level.[48,49] IV iron treatment should be thus a primary form of therapy in case of severe anemia (hemoglobin $<$10 g/dL), active disease (CRP $>$5 mg/L), need for a rapid Hb recovery, and while treating with erythropoietin agents.[47,50] Notably, parenteral iron replacement therapy improves QOL more efficiently than oral iron supplementation.[47] Interestingly, Çekiç et al[51] observed that a greater increase of QOL occurs in patients with ulcerative colitis than with CD.

The first IV iron preparations have carried iron in oxyhydroxide form, implicating its toxicity. High-molecular weight iron dextran (HMWID) was introduced in 1954 and allowed implementation of higher doses to the therapy, but the risk of

anaphylaxis was also increased. The incidents of severe side effects decreased in 1991, when low-molecular weight iron dextran preparations were developed. Consequently, studies involving the effectiveness and safety of low-molecular weight iron dextran in patients with IBD were performed both in adults and in children.[52–54] Regarding adult patients, the anaphylactic reactions were observed in 2% to 6% of all cases studied.

An additional reduction of severe adverse effects could be achieved by premedication with diphenhydramine and acetaminophen before iron dextran infusion.[55] Dextran-free iron preparations have become available in 1999 and 2000, when ferric gluconate and iron sucrose (IS) were discovered, respectively. Iron sucrose is currently in a phase 4 clinical trial (NCT01067547) in patients with IBD. Studies performed over the last 10 years exhibited efficacy, high safety profile, and wide availability of IS, and since then, IS has become standard treatment of IDA in IBD.[56–62]

In the last few years, newer IV iron formulations, iron carboxymaltose, iron ferumoxytol, and iron isomaltoside, were reported. These new preparations do not require test dosage before starting the treatment because of the reduced risk of releasing free iron quantities to the blood and lack of acute toxicity.[63] New parenteral iron preparations may be administered at higher doses because they are safer than traditional iron formulations (low-molecular weight iron dextran, ferric gluconate, and IS), and the treatment duration is shortened. Currently, most of these new IV iron formulations are still in clinical trials in patients with IBD. For instance, phase 3 clinical trials are presently performed to estimate the administration regimen of ferumoxytol in patients with IDA, also in subgroup of patients with IBD (ClinicalTrials.org identifier: NCT01114139, NCT01114217, and NCT01114204). Similarly, a Phase 3 study is currently underway to evaluate the use of iron isomaltoside in patients with IBD and IDA (NCT01599702).[64] Ferric carboxymaltose is the only novel IV iron preparation, which has already been approved for treating patients with IBD in Europe.[65,66] Namely, Kulnigg et al[66] in a randomized study demonstrated the efficacy and good tolerance of carboxymaltose in comparison with oral iron preparations. Noteworthy, Evstatiev et al compared ferric carboxymaltose and IS in a multinational randomized study and demonstrated the superiority of this new formulation in treating anemia in IBD versus IS. In addition, more patients, who were treated with ferric carboxymaltose, achieved either 20 g/L increase in hemoglobin level or normalization of Hb level compared with IS group.[65]

PATHOPHYSIOLOGY OF ANEMIA OF CHRONIC DISEASE

The development of anemia of chronic disease is complex and seems to be a result of immune system mobilization. Three mechanisms have been known to play a role in ACD: iron reallocation off the serum, impaired proliferation and further maturation of erythroid progenitors, and reduced life span of erythrocytes.[67] Several cytokines have been proven to influence these mechanisms, with 4–interleukin (IL)-1, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and IL-6–being the main focus. The fact that TNF-α inhibits erythropoiesis has been first reported in late 1980s and ever since the efforts have been made to explain the molecular basis of this phenomenon.[68,69] Briefly, proper maintenance of erythropoiesis is possible due to an appropriate balance between transcription factors GATA-1, GATA-2, and PU.1.[70–72] GATA-2 is substantial during proliferation of immature hematopoietic progenitors, whereas GATA-1 maintains antiapoptotic function on erythroid progenitors and coordinates terminal differentiation into red blood cells.[71] TNF-α influences erythropoiesis in both indirect and direct manner. The cytokine works indirectly through the suppression of erythropoietin synthesis in kidneys, potentially by GATA-2 and nucleus factor kappa B overexpression.[73–75] Lowering erythropoietin levels inhibits erythropoietin receptor-dependent signaling pathways, which are followed by GATA-1 downregulation.[76] However, there is a direct influence of TNF, likely to be mediated through TNF receptor (TNFR1), which has been investigated in cell lines.[77] Inter alia, reversal of GATA-1/GATA-2 expression in favor of GATA-2 and PU.1 overexpression, which is a known GATA-1 repressor, can thus be observed.[70,78] Furthermore, increased levels of GATA-2 facilitate megakaryopoiesis while suppressing the erythroid proliferation.[79] Direct, repressive effect of nucleus factor kappa B on erythroid genes was also suggested.[80]

Libregts et al[81] observed that anemia develops in CD-70 transgenic mice with enhanced IFN-γ production and attempted to investigate this issue. First, the reduced lifespan of erythrocytes was documented because of an increased erythrophagocytic capacity in spleen. Moreover, erythropoiesis in this mouse model was impaired, which was further explained as an increase of PU.1 level through interferon regulatory factor-1. Further in vitro studies indicated that IFN-γ triggers apoptosis in erythroid progenitors because of induction of proapoptotic molecules, such as TRAIL or TWEAK.[82,83] Taken together, these findings indicate the potential role of IFN-γ in developing ACD.

Finally, the pathophysiology of ACD is affected by the mechanisms underlying iron sequestration. The key role is played here by hepcidin, a peptide produced principally in the liver.[84] Hepcidin serves as a ligand for cellular iron efflux receptor, ferroportin, which exports iron into the plasma and is present on various cell membranes, intestinal epithelial cells, macrophages, hepatocytes, and placental cells. Binding of hepcidin to ferroportin results in internalization and proteolysis of the latter; thus, the iron is trapped inside the cells, out of reach of erythropoiesis.[85] The regulation of hepcidin is versatile and coordinated by several mediators and enzymes, which are summarized in Figure 2.[14] A more detailed physiology of hepcidin will be described in the next chapter.

Hepcidin and Its Role in IBD

Hepcidin is produced as a preprohormone consisting of 84 amino acids and later converted to a 25-amino acid hormone; both are detectable in serum and urine. Hepcidin belongs to the defensins family—antimicrobial proteins serving as natural antibiotics, what

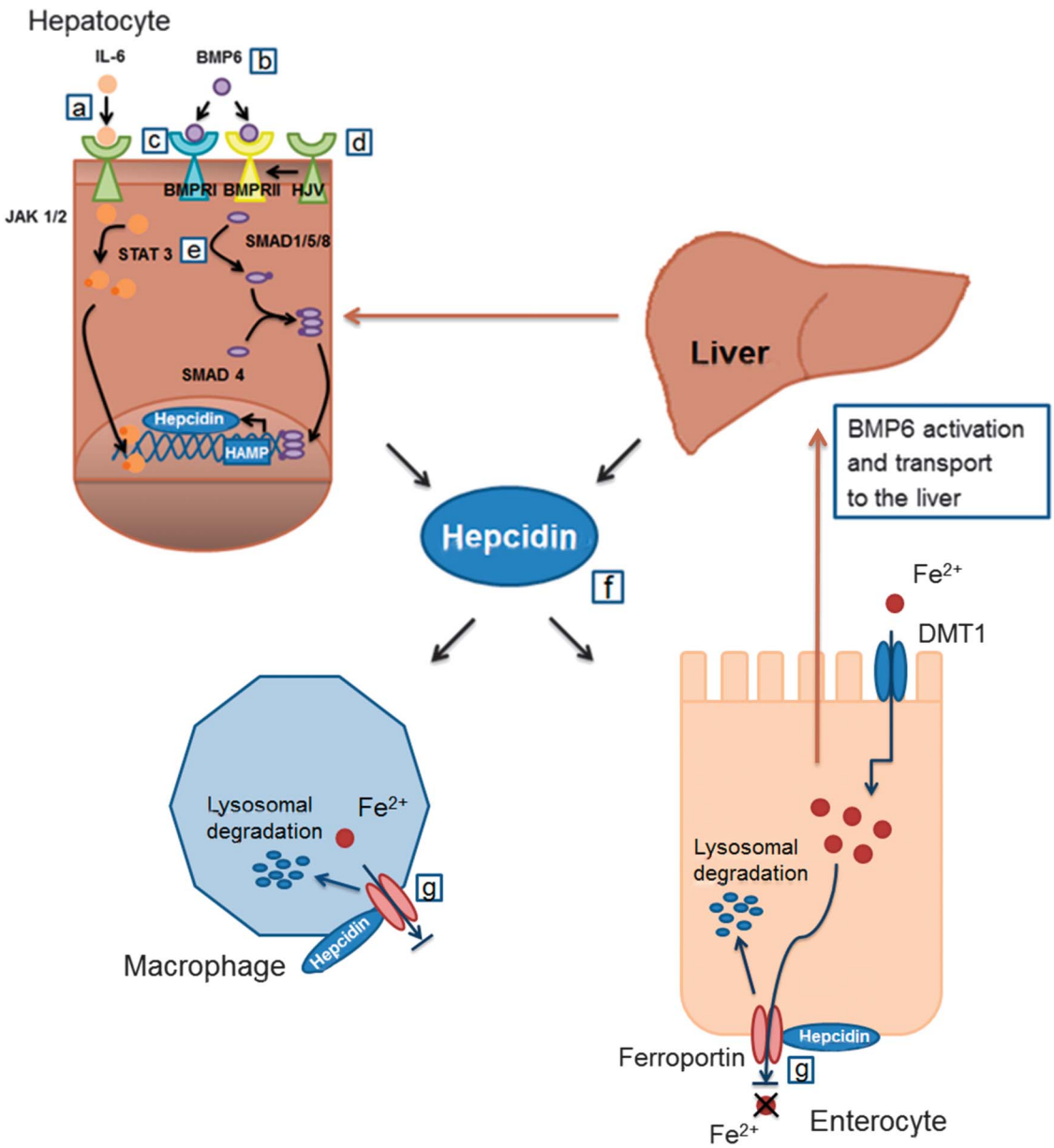

FIGURE 2. Role of hepcidin in regulation of iron homeostasis in patients with IBD. Hepcidin is a general regulator of iron homeostasis. During inflammation, proinflammatory cytokines, especially IL-6 (as a result of JAK-dependent activation of STAT3), increase hepcidin gene expression. Hepcidin production is also regulated by enteric iron stores. Absorption of iron from the intestinal lumen into enterocyte through DMT1 activates BMP6 production; BMP6 travels to the liver where it binds to the BMP receptors 1 and 2 (BMPRI and II) and the coreceptor HJV. This results in phosphorylation of SMAD1, SMAD5, and SMAD8 and complex creation with SMAD4. After translocation to the nucleus, the complex activates the HAMP gene promoter and hepcidin is synthesized. Hepcidin circulating in serum binds to ferroportin, what leads to lysosomal degradation of the latter and reduction of iron release from enterocytes and macrophages. Hepcidin may also directly inhibit DMT1. Letters from a to g indicate specific targets for novel drugs, mentioned in the review: b anti-bmp, heparins, a tocilizumab, c LDN-193189, d sHJVFc, e AG490, PpYLKTK, f monoclonal antibodies, anticalins, and spiegelmers, g antibody for ferroportin (LY2928057), fursultiamine. Adapted from Ref. 14 modified. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

implies the increase in their production during infection.[86,87] Apart from the inflammatory state, also iron and erythropoietic activity are believed to play a role in regulation of hepcidin expression. These regulatory mechanisms mainly occur at the transcriptional level.

Inflammation-dependent signals regulating hepcidin involve IL-6 and Bone Morphogenetic Protein (BMP) receptor.[88,89] In addition, a recent study by Wallace and Subramanian[90] showed IL-22 to induce hypoferremic state and hepcidin

expression in mice after stimulation with lipopolysaccharide (LPS); however, the correlation does not seem to be as strong as in the case of IL-6. Of note, hepcidin level can also be elevated in noninfectious inflammatory disease, such as malignancies, chronic kidney disease, and various autoimmune diseases.[91–93]

Importantly, hepcidin and related cytokines were found crucial in pathophysiology of anemia in IBD. Although its role has not been yet fully elucidated, new concepts have emerged just recently. Noteworthy, both hepcidin, its prohormone and different derivatives, and isomers have been studied. To begin with, Basseri et al[94] found a strong correlation between hepcidin and IL-6 levels in 17 patients with CD. The study also showed that hepcidin measured in urine could be related to plasma-derived one. Moreover, hepcidin level significantly correlated with ferritin level. Of note, Oustamanolakis et al[95] demonstrated on a large group of patients that serum prohepcidin is not consistent with hepcidin and significantly correlates only with hemoglobin. Moreover, in a multivariate analysis, hepcidin, but not prohepcidin level, significantly correlated with ferritin and disease activity in patients with ulcerative colitis. Poor association between prohepcidin and CRP was further confirmed by Nagy et al.[96] In a recently published study, patients were grouped by the presence of anemia, disease activity, and the level of ferritin. Although hepcidin showed excellent correlation with ferritin in nonlowered ferritin anemic patients with active IBD, again it showed no association with inflammatory markers (CRP and activity indices).[97] In contrast to these findings, interesting results were collected from 19 pediatric patients with CD. Patients with active disease exhibited higher levels of urine hepcidin. Of note, it was found that the cutoff value of IL-6 at 5 pg/mL can be used to asses disease activity. Furthermore, patients were given ferrous sulfate orally, and blood samples were taken hourly for 4 hours to determine iron level. Patients with active disease demonstrated lower increase in iron concentration compared with those with inactive disease.[98] These observations lead to the observation that inflammation plays an indisputable role in anemia management, as it impairs intestinal iron absorption. Therefore, iron forms other than oral should be taken into consideration when treating anemia in IBD. However, research on a larger group of patients and at different age is needed.

Finally, interesting findings were provided by Shanmugam et al.[99] Using rodent models of colitis, through cohousing and fecal transplantation, they demonstrated that microbiota possesses the ability to modulate hepcidin expression in the liver. With an emerging role of intestinal dysbiosis in IBD etiology and growing interest in manipulating the microbiota for therapy purposes, this could bear a plausible treatment option. However, the observed phenomena need further investigation.

Standard Therapy for ACD in IBD

Treating ACD is not a trivial issue and, contrary to IDA management, iron therapy is not the first-line approach in ACD, which is likely due to impaired iron absorption, as mentioned above. IV preparations are obviously superior to oral in states requiring rapid Hb increase; nevertheless, the nature of ACD calls for special care. For example, patients exhibiting functional iron deficiency who are chronically treated with IV iron could demonstrate iron overload toxicity in future. Thus, patients with refractory or relapsing anemia should be selected from anemic IBD population and treated in a more individual manner.

Although persistent inflammation plays a pivotal role in maintaining ACD, controlling inflammatory state is an important step in IBD-related anemia. However, controlling the course of chronic disease is often unachievable. Moreover, FERGImain study showed that even in nonanemic patients with quiescent disease activity who received placebo anemia recurred in 39.4% after 8 months.[100] Such high recurrence rate could depend on subclinical inflammation persisting in intestinal mucosa. Also, it should be kept in mind that clinical indices used for assessing disease activity occasionally fail to genuinely reflect it. In this case, a new way of assessing inflammatory score in patients with IBD, through mucosal healing, could be a promising scoring system.[101]

Biological agents targeting inflammatory cytokines are being used more frequently in treating IBD. It was shown that the anti-TNF-α therapy improves hematologic markers in treated patients.[102,103] In addition, anti-TNF-α agents negate the cytokine influence on erythropoiesis enhancing erythropoietin production and erythroid genes expression. Also by inducing mucosal healing, they reduce blood loss and production of proinflammatory cytokines, therefore inhibiting hepcidin production.

Other treatment would include the use of erythropoietic agents. This approach has been introduced and recommended already in the first guidelines created by Gasche et al, and no exceptions were made in the latest ones. The erythropoietic agents are reserved as a second-line treatment for patients who do not respond satisfactorily to IV iron in whom disease activity is adequately controlled. However, as the chronic use of erythropoietic agents could worsen the functional iron deficiency, it is usually combined with IV iron.[104–106] Of note, the latest study conducted by Liu et al[107] showed the potential of a combination of erythropoietin (EPO) and enteral nutrition, containing a small amount of iron element to treat anemia in patients with IBD. Consequently, anemic patients could benefit from receiving iron in a more physiological pattern than directly by the IV solutions. Larger studies are needed to address the pros and cons of this issue.

NOVEL TREATMENT OPTIONS FOR ACD

Although there are several treatment options for ACD available, none of them seems perfect, and new potential drugs targeting hepcidin are in development. They could be an advantage for patients who failed to respond to EPO treatment, for the main cause of the failure is high hepcidin concentration. Currently, hepcidin antagonists could be split into 3 groups: hepcidin production suppressors, peptides which neutralize hepcidin, and hepcidin-ferroportin interfering agents.

Hepcidin Production Suppressors

Search for a novel ACD medication began with BMP pathway inhibitors. First, recognized target was hemojuvelin

(HJV), an essential coreceptor for BMP/SMAD signaling. Soluble HJV.FC (sHJV.FC) comprised the extracellular domain of HJV fused to the Fc portion of human IgG, inhibited hepcidin expression, and increased ferroportin expression, intestinal iron absorption, and serum iron level in mice.[108] It also blocked IL-6–mediated hepcidin induction in the same study. Another possible target is the phosphorylation of type I BMP receptor. Although dorsomorphin-derivative LDN-193189 showed similar outcomes to sHJV.FC in treating anemia in ACD rodent model, it was less selective than the latter because of possible off-target effects.[109] Eventually, efforts have been made to develop anti-BMP antibody, which would block the interaction between BMP and its receptor. To conclude, all 3 treatment options mentioned above effectively ameliorated disease severity, intestinal inflammation, and iron stores in mice with T-cell transfer colitis[110].

Heparins are yet another treatment option based on attenuation of BMP pathway signaling. They possess strong ability to inhibit hepcidin expression, possibly by BMP6 sequestering and blocking SMAD signaling.[111] Interestingly, the major activity of heparin–anticoagulant would remain desirable in selected cases. A study on a large cohort in Taiwan showed a significantly higher risk for deep vein thrombosis and pulmonary embolism in hospitalized patients with IBD.[112] Thus, patients with IBD and anemia who are at a greater risk of thrombotic events could benefit from heparin therapy. Nevertheless, synthetic heparins with weaker anticoagulant activity are being tested for a wider usage.[113,114] Studies confirmed their inhibitory effect on hepcidin expression in the rodent model of inflammation.[113,114]

Although the inflammatory pathway incorporating IL-6/STAT3 axis is involved in hepcidin upregulation, blocking this pathway is a possible treatment of ACD. Tocilizumab, the anti-IL-6 receptor antibody, improved anemia in collagen-induced colitis in monkeys and reduced hepcidin expression.[115] Other agents include the inhibitor of STAT3 phosphorylation, AG490, or PpYLKTK, which disrupts STAT3 dimerization.[116,117]

Antihepcidins

Decreasing the burden caused by hepcidin could possibly lay in neutralizing it, either by direct blockage or sequestration. To date, monoclonal antibodies, anticalins, and spiegelmers are best candidates for the antihepcidin treatment.

An antihepcidin antibody improved the anemia in a rodent model of ACD caused by heat inactivated *Brucella abortus* only when coadministered with erythropoiesis stimulating agent (ESA).[118] A fully humanized monoclonal antibody, LY2787106, has already passed a phase I study in patients with cancer-related anemia, but further data remain unknown (NCT01340976).

Hepcidin binding could also be executed by anticalins and molecules derived from human lipocalins, which transport small hydrophobic moieties. By adjusting the affinity and selectivity, an antihepcidin therapeutic PRS-080 has been obtained. PRS-080 efficiency was shown on cynomolgus monkeys, where it caused effective iron mobilization.[119]

Spiegelmers are synthetic oligonucleotides designed to bind and neutralize diverse molecules. Owing to their L-enantiomeric structure, they are nuclease resistant, stable, and generally neutral to the immune system. A spiegelmer for antihepcidin use, NOX-H94, has recently passed phase II study in patients with cancer-related anemia; however, the results obtained in the study have not been revealed yet (NCT01691040).

Interfering the Ferroportin–Hepcidin Interaction

A monoclonal antibody targeting ferroportin prevents hepcidin from binding to the transporter without affecting the iron efflux. Newly designed humanized agent, LY2928057, exhibited promising results on cynomolgus monkeys, and phase I trials are on their way for this drug.[120]

Fursultiamine is the model drug relying on sequestration of hepcidin binding domain in ferroportin, which contains the sulfhydryl residue.[121] However, pharmacokinetics of fursultiamine is not satisfactory and therefore similar agents, but with a minor modification, could be a good alternative.

CURRENT DIAGNOSTIC PROCEDURES FOR ANEMIA IN IBD

The diagnosis of anemia in IBD should arise from the most basic laboratory tests to the more specific for different states of low hemoglobin in serum. To begin with, the clinician should follow the regular hematological algorithm for anemia investigation. Full blood count with mean corpuscular volume, mean corpuscular hemoglobin, and reticulocyte count need to be evaluated. The volume of erythrocyte directs the approach toward more precise causes whether it is micro-, macro- or normocytic anemia. The most difficult to come up against is to differentiate iron deficiency and ACD, the 2 most frequent types of anemia. Various guidelines and methods of proceedings suggest that the diagnosis should be guided by serum ferritin concentration. Accordingly, IDA is likely if the ferritin is below 30 μg/L, and ACD can be diagnosed when ferritin is above 100 μg/L. However, such extremities are rarely seen in everyday clinical practice making diagnosis questionable. Clinicians should also be aware of ferritin being an acute phase protein, making it less valuable in assessing anemia during disease flares.

Soluble transferrin receptors (sTfR) and sTfR-ferritin (sTfR-F) are promising candidates for such markers. Iron in serum is transported by transferrin, which binds to the receptor on cellular surface causing internalization of the complex. In acidic environment of endosomes, iron unbinds and the remaining Tf-TfR complex returns to the cell membrane, where the apotransferrin is released. After the process, soluble form of TfR (sTfR) could be detected in serum. It has been proven that sTfR concentration relates closely to the global level of cellular TfR, whereas the expression rate of the latter is directly proportional to erythroid activity.[122,123] Although transferrin is a negative acute phase reactant, concentration of soluble forms of its receptor in

serum is not affected by inflammation.[124] Although no superiority to ferritin was shown when measured alone, the incorporation of ferritin and sTfR into 1 index (sTfR/logF) improved sensitivity and specificity drastically.[125] For example, the index succeeded to differentiate coexisting iron deficiency with ACD in a group of 96 patients with rheumatoid arthritis.[126] Moreover, the efficacy of sTfR/logF was observed in a group of 100 patients with IBD, and no correlation was found either with CRP levels or the disease activity.[127] However, the ability to discriminate subgroups deficient in iron from ACD groups in IBD has yet to be evaluated. The addition of the serum sTfR and sTfR/logF index assessment could facilitate the identification of the iron deficiency in individuals, especially the concurrent IDA in patients with IBD.

Hemoglobin content in reticulocytes (CHr) or reticulocyte hemoglobin equivalent (Ret-He) was proposed as an early iron-deficient erythropoiesis marker.[128] Classical erythrocyte parameters including hemoglobin (HGB), mean corpuscular volume, and mean corpuscular hemoglobin describe mature red blood cells, which have a 120-day lifetime, allowing the detection of iron deficiency relatively late. In contrast, reticulocytes circulate in the serum only for 1 to 2 days making CHr a good and rapid measurement of iron availability for erythropoiesis. Also, CHr is independent of acute phase reactivity. In a study by Marković et al,[129] patients with concurrent IDA and inflammation exhibited decreased CHr levels, whereas in patients with uncomplicated ACD, the marker was within the control group range. Percentage of hypochromic red cells (%Hypo-He) demonstrates the portion of mature erythrocyte with inadequate hemoglobinization and may increase before changes in mean values indices are seen. Again, as it remains unaffected by inflammation, %Hypo-He shows reliability in finding iron deficiency in patients with ACD.[130] Unfortunately, the study was comprised heterogeneous individuals who had diverse entities, and therefore, further trials in patients with IBD examining both above-mentioned parameters are needed.

The quantification of hepcidin could serve as a support to traditional hematological markers. Indeed, in a study composed by Enko et al,[131] hepcidin assessed in patients with CRP >5 mg/L was significantly increased compared with patients without acute phase reaction. Nevertheless, hepcidin was only slightly better in prediction of functional iron deficiency than ferritin in case of patients with CRP >5 mg/L. Both parameters were inferior to sTfR and sTfR-F index. These findings are consistent with a recent study, where mass spectrometry evaluation of hepcidin assay failed to reflect superiority to ferritin in diagnosing infective dose in elderly patients presenting mixed anemia.[132] Hepcidin measurement had the same limitations in discriminating infective dose from patients with mixed anemia as ferritin and transferrin, whose levels are predicted to change during disease flare-up. Also, it is more expensive and does not focus directly on the iron management, making it rather unsuitable for everyday practice.

Anemia in IBD often recurs, what obligates the physician to screen patients for this complication every 3 months. Screening should thus consist of a broader list of available parameters beside hemoglobin, the level of which decreases after loss of large portion of total body iron. Full blood count, ferritin, transferrin saturation, and disease activity assessment should be used and followed by more specific tests. Macrocytic anemia is usually paired with hypovitaminosis, and therefore, vitamin B12 and folic acid levels should be checked. However, anemia associated with high mean corpuscular volume could seem as a treatment complication with immunosuppressive drugs. When the myelosuppression is suspected, bone marrow smear and differential white blood cell count need to be performed, even in patients not receiving such treatment to exclude myelodysplastic syndrome.

Latest data suggest possible association between iron deficiency and increased platelet counts in patients with IBD.[133] This may lead to the potential beneficial effect of conscientiously performed screening. Detecting the iron deficiency before the anemia develops could shorten the therapy, lower the costs, and prevent the possible thrombotic events in these patients.[134] Not to mention the convenience of patients, which would be brought by such approach.

CONCLUSIONS AND INFERENCES

Anemia is a very common finding among patients with IBD, regardless of its cause. Anemia impairs patient's QOL and increases mortality; thus, the proper approach at each step of its management is extremely important. First, confirmation of the diagnosis using adequate laboratory indices is substantial to differentiate between the diverse types of anemia, and novel indices, which could facilitate the process in ambiguous cases, are particularly valuable. Furthermore, initiation of the treatment should go hand in hand with normalization of IBD activity. Last but not least, monitoring for recurrent anemia and preventive treatment is essential in the course of therapy. Such a "pro-active" concept of anemia management, taking into consideration, all these steps could not only improve the QOL of patients with IBD but is also economically beneficial.

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APPENDIX 1

Total body iron deficit is used to estimate total parenteral iron dose needed; currently, Ganzoni's formula is recommended as a gold standard to calculate total body iron deficit[135]:

$$\text{Iron deficit (mg)} = \text{Body weight (kg)} \times (\text{Target hemoglobin} - \text{Actual hemoglobin [g/dL]}) \times 2,4 + \text{Stored iron (500 mg)}$$

However, the Ganzoni's formula is prone to errors; for example, it may underestimate the amount of stored iron in male individuals (correctly estimated at 700–900 mg instead of 500 mg).[136] The formula is also cumbersome and inconsistently used in clinical practice.[64,66]

Kulnigg et al in the FERGIcor trial proposed a novel, simple regimen to predict individual iron requirements for ferric carboxymaltose. Baseline hemoglobin level and body weight are sufficient to calculate the iron need. Following this scheme enables a more effective and safer treatment of IDA in patients with IBD than doses calculated based on Ganzoni's formula.[65]

Baseline Hb (g/dL)	Body Weight <70 kg	Body Weight ≥70 kg
10–12 (women)	1000 mg	1500 mg
10–13 (men)		
7–10	1500 mg	2000 mg

Although only a simple scheme was used to estimate the dose for ferric carboxymaltose in FERGIcor trial, it may also be used in clinical practice for dosing other IV iron preparations.[137]