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Review

Title: Current evaluation and management of anaemia in patients with inflammatory bowel disease

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Abbreviations

ACI, anaemia of chronic inflammation; AIHA, autoimmune haemolytic anaemia; CD, Crohn's disease; CHr, reticulocyte haemoglobin; CRP, c-reactive protein; ECCO, European Crohn's and Colitis Organisation; EPO, erythropoietin; ESA, erythropoietin stimulating agent; FA, folic acid; FCM, ferric carboxymaltose; FID, functional iron deficiency; Hb, haemoglobin; Hcy, homocysteine; HMWID, high molecular weight iron dextran; IBD, inflammatory bowel disease; ID, iron deficiency; IDA, iron deficiency anaemia; i.v., intravenous; LMWID, low molecular weight iron dextran; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; MDS, myelodysplastic syndrome; PLP, pyridoxal phosphate; RBC, red blood cell; RDW, red cell distribution width; RES, reticuloendothelial system; RPC, restorative proctocolectomy; SRL, serum retinol levels; sTfR, soluble transferrin receptor; TfR/F, transferrin-ferritin ratio; TSAT, transferrin saturation; tHcy, total homocysteine; TIBC, total iron binding capacity; UC, ulcerative colitis; ZPP, zinc protoporphyrin; %HYPO, percentage of hypochromic red cells

Abstract

Introduction: Anaemia is a common extraintestinal manifestation in IBD patients and considerably impacts disease prognosis, hospitalisation rates and time lost from work. While iron deficiency anaemia is predominant, combinations of haematimetric and biochemical markers enable detection and targeted therapy of other aetiologies including vitamin B12/folic acid deficiencies, haemolysis, myelosuppression and pharmacotherapies.

Areas covered: Current literature was searched for articles focusing on aetiology, diagnostics and therapy of anaemia in IBD. In the light of their own experience, the authors describe the physiology of anaemia in IBD and present current evidence endorsing diagnostic and therapeutic options, focusing particularly on non-iron-related aetiologies**.**

Expert commentary: Anaemia in IBD is polyaetiological, reaching far beyond iron deficiency anaemia. While clinicians need to be aware of the increasing pallet of diagnostic tools and therapeutic options, detailed studies are needed to develop more convenient test procedures, long-term treatment and monitoring strategies, and unified guidelines for daily practice.

Keywords: Anaemia, Inflammatory bowel disease, iron deficiency, disease management, diagnostic procedures

1. Introduction

Anaemia is the most common systemic complication and extraintestinal manifestation of inflammatory bowel disease (IBD), both at diagnosis and during disease[1, 2]. Anaemia has a substantial impact on quality of life in IBD patients, affecting physical, emotional and cognitive functions, ability to work, rate and duration of hospitalisation, and healthcare costs[3]. Thus, anaemia in IBD is not just a laboratory marker; it is a disease complication that demands appropriate diagnostic and therapeutic management[4].

Based on the World Health Organisation (WHO) definition of anaemia[5] (Table 1), large community studies from both the USA and Europe have reported prevalences of 6%–74% in historical cohorts of hospitalised IBD patients[6-9]. Recent studies of IBD outpatients in Switzerland and Scandinavia, using population-based datasets, found the prevalence of anaemia to be 19%-21%[10, 11]. While two-thirds of IBD patients have anaemia at diagnosis, its prevalence and causes may change during follow-up. These substantial variations may be due to differences in study population (e.g. age, disease activity) as well as in the definition of anaemia.

In children, anaemia is even more common (approx. 70%) than in adults (30–40%)[12]. Data describing the prevalence of anaemia in the elderly IBD population are scarce[13]. Filmann et al. calculated the overall prevalence of anaemia in patients with Crohn's disease (CD) to be 27%, compared with 21% in patients with ulcerative colitis (UC). Fifty-seven percent of anaemic patients were iron deficient. Age and gender-stratified basis prevalence showed that whereas male patients aged 30–64 years have a significantly lower risk for anaemia than younger patients (<30 years) or men older than 65 years, the prevalence in women is almost entirely independent of age. Gender was not significantly associated with the existence of anaemia[14]. Gomollon and Gisbert calculated a mean prevalence of 16% in outpatients and 68% in hospitalised patients[15]. These data were confirmed by a more recent study of a Swedish cohort showing that the prevalence of anaemia was significantly higher in hospitalised UC and CD patients compared to the outpatient population[16]. Furthermore,

anaemia was more frequent in CD than in UC patients[16, 17].

Since normal haemoglobin levels vary with age, gender and ethnicity, there is increasing debate as to the exact haemoglobin (Hb) levels which should be applied to define anaemia in general, and particularly in the elderly population. For example, the lower limits of normal haemoglobin concentration are lower in African Americans (11.5 g/dL for women, 12.9 g/dL for men) and likewise in the elderly. Interpretation of haemoglobin and haematocrit levels needs to take account of such modulating factors[4, 18].

2. Pathogenesis

The cause of IBD-related anaemia is multifaceted. The two most frequent aetiologies by far are iron deficiency anaemia (IDA), and anaemia of chronic disease (ACD). In the majority of cases, however, IBD-associated anaemia is a prime example of combined IDA and ACD[19]. While vitamin B_{12} and folate deficiency-associated and drug-induced anaemia (sulfasalazine[20], thiopurines[21], methotrexate[22], calcineurin inhibitors[23]) are less widespread, these possibilities should also be considered. Rare aetiologies of anaemia in IBD include deficiencies of vitamins D, A and B_6, autoimmune haemolytic anaemia and myelosuppression (Table 2).

2.1 Iron deficiency anaemia (IDA)

Iron deficiency (ID) is the most common cause of anaemia in IBD, with a reported prevalence of approximately 55% in IBD patients and 88% of children with IBD. At diagnosis, 90% and 95% of children with CD and UC, respectively, were found to have iron deficiency[24]. ID arises due to blood loss through ulcerations of the intestinal mucosa (especially in UC patients), reduced dietary intake (see below) and decreased intestinal iron absorption (for review, see[19]). Inflammatory cytokines (IL-1, IL-6, oncostatin M) can impair intestinal iron absorption via hepcidin-induced ferroportin degradation independently of disease location and underlying IBD type[25]. In addition, duodenal iron absorption has been shown to be inhibited by TNF via a hepcidin-independent mechanism based on TNF-induced iron storage within ferritin in enterocytes both *in vitro* and *in vivo*[26, 27].

2.2 Anaemia of chronic disease

Anaemia of chronic disease (ACD), also referred to as anaemia of chronic inflammation (ACI), and first described by Cartwright in 1946[28], is characterised by mild to moderate anaemia (Hb <8g/dL is rare), normal or reduced mean corpuscular volume (MCV), reduced serum iron, normal to elevated serum ferritin, and reticuloendothelial system (RES) stores that are elevated relative to total body iron, occurring in the setting of infection, inflammatory disease or malignancy[29]. The exact prevalence of ACD in IBD is unknown.

Recent advances in the molecular understanding of ACD based on the combined action of hepatocyte- and macrophage-derived hepcidin and inflammatory cytokines may be summarised as follows (for review see[30, 31]):

Firstly, cytokine (IL-1, IL-6)-stimulated hepcidin induces ferroportin degradation, leading to impaired intestinal iron absorption and iron release from RES, trapping iron in macrophages, hepatocytes and enterocytes: Erythropoiesis is therefore *iron restricted;*

secondly, erythrophagocytosis is enhanced by cytokine (TNF, IL-1, IL-6, IFN-γ)-activated macrophages, resulting in shortened erythrocyte lifespan;

thirdly, erythropoiesis is suppressed due to the direct action of TNF, IL-1, IL-6 and IFN-γ in the bone marrow, exerting pro-apoptotic effects on the earliest erythroid-committed precursor BFU-e (burst-forming unit-erythrocyte), and also as a result of impaired erythropoietin (EPO) production due to direct inhibition by the EPO promotor. Moreover, cytokines have been shown to interfere with the EPO signalling process and downregulate EPO receptors, thus inducing “EPO-resistance” in erythroid progenitor cells (Figure 1).

2.3 Vitamin B_{12} *deficiency*

Up to 22% of patients with Crohn’s disease suffer from vitamin B_{12} deficiency if diagnosis is based on serum levels. Causes include ileal disease or resection, fistulas, small bowel bacterial overgrowth and reduced intake. Based on their systematic review including 42 articles, Battat et al. concluded that B_{12} deficiency in CD patients with no ileal resection (or resection of <20cm) is no more common than in the general population. Only ileal resection greater than 30cm predisposes to B_{12} deficiency and the need for lifelong B_{12} replacement,

while up to 48% of patients with shorter resection lengths of 20–30cm are at risk of eventually developing B_{12} deficiency[32].

In contrast, the prevalence of B_{12} deficiency in UC has generally been found to be comparable to that of the general population, except in patients who have undergone restorative proctocolectomy (RPC) with ileoanal pouch anastomosis. Three possible causes are under discussion: *first*, reduced absorptive capacity for B_{12} due to resection; *second*, bacterial overgrowth (a well-known cause of vitamin B_{12} deficiency) in both the small bowel reservoir and the more proximal ileum due to ileoceocal valve removal and changes in transit time; and *third,* dietary intolerance[32, 33].

2.4 Folic acid deficiency

As a coenzyme for cellular one-carbon metabolism, folic acid (FA) plays a crucial role in biological methylation and nucleotide synthesis, the latter being essential for complete development of erythrocytes and deoxyribonucleic acid (DNA).

Although less prevalent today than previously reported in historical IBD cohort studies, FA deficiency still appears to be relatively common in CD, with a prevalence ranging from 16%-28%, compared to 1.4%-12% in UC[34, 35]. While FA deficiency is a known, common side effect of sulfasalazine[36] and methotrexate therapy[37], inadequate dietary intake, disease activity and ileum involvement have also been shown to be risk factors. Up to 8% of CD patients have macrocytic anaemia[38]. In this context, it should be kept in mind that FA deficiency may arise as a consequence of vitamin B_{12} deficiency, since vitamin B_{12} plays a role in the conversion of inactive methyltetrahydrofolic acid to active tetrahydrofolic acid[39].

Notably, almost all studies to date have used serum folate level rather than red blood cell (RBC) folate level, which may be the more accurate test, as it reflects tissue FA status over the lifetime of red cells. RBC folate level is therefore regarded as an indicator of longer term FA status than serum FA[39]. Although FA has been shown to be a stronger determinant of total homocysteine (tHcy) levels than vitamin B_6 and B_{12}, plasma tHcy should be used to

confirm suspected FA deficiency only in unclear situations; a level of 15µmol/L indicates FA deficiency, if vitamin B_6 and B_{12} deficiencies are excluded[39].

2.5 Vitamin D deficiency

In the context of the high prevalence of vitamin D deficiency in the IBD population[40, 41], new insights into the pathogenesis of anaemia have been gained from studies in patients with chronic kidney disease and in children[42]: Vitamin D status (serum concentration of the prohormone 25-hydroxyvitamin D [25(OH)D]) correlates inversely with the prevalence of anaemia and resistance to erythropoiesis-stimulating agents (ESAs), and directly with blood haemoglobin levels. In addition, vitamin D repletion in anaemic haemodialysis patients has been shown to correlate with lower ESA requirements[43]. More recently, in a pilot study of healthy volunteers, Bachetta et al. demonstrated that oral vitamin D replacement resulted in a significant decrease in circulating levels of hepcidin within 24h after supplementation[44].

2.6 Vitamin A deficiency

The interaction between vitamin A, iron status and anaemia has been widely reported[45]; however, the exact mechanism involved in this interaction has not been well characterised. Vitamin A has been shown to modulate not only different steps of erythropoiesis (e.g. upregulation of renal erythropoietin expression)[46], but also iron homeostasis, influencing iron mobilisation mechanisms and intestinal iron absorption through upregulation of iron regulatory protein-2[47] expression and induction of FPN1 gene expression.

Thus, in the light of the evidentially high prevalence of vitamin A deficiency in IBD patients[48], vitamin A deficiency may be presumed to cause, or at least aggravate, anaemia in IBD. From a diagnostic point of view, it is important to bear in mind that the use of serum retinol levels (SRL) as a diagnostic parameter, as practised in almost all trials, may underestimate actual vitamin A deficiency. Since SRL poorly reflects vitamin A status, the relative dose response (RDR) test, an accurate, indirect indicator of hepatic vitamin A stores, has been recommended as a better diagnostic tool[48]. Two studies in children with poor vitamin A status

demonstrated that vitamin A supplementation increases erythropoietin and haemoglobin levels[49]. Studies in the IBD population are still lacking.

2.7 Vitamin B_6 deficiency

Vitamin B_6 (pyridoxine) is a water-soluble vitamin that comes in several forms, pyridoxal phosphate (PLP) being its active form. Pyridoxine acts as a cofactor of 5-aminolevulinic acid synthase (ALAS), the first enzyme of the haem biosynthesis pathway. Thus, vitamin B6 deficiency results in microcytic anaemia[50]. Anaemia attributable to vitamin B_6 deficiency, first described by Maier in 1957[51], is now well known in a range of clinical conditions[52, 53]. Recently-published data from three small studies demonstrated vitamin B_6 deficiency (defined by serum PLP levels <10nmol/L) in 10%–30% of IBD patients, one study additionally demonstrating a greater risk in patients with active disease compared with those with quiescent disease[54, 55].

2.8 Autoimmune haemolytic anaemia and myelosuppression

Autoimmune haemolytic anaemia (AIHA) is a rare type of anaemia which occurs in 0.2%-1.7% of UC patients, according to recent reports[56]. AIHA can occur either prior to or after manifestation of UC. While AIHA primarily occurs due to the development of erythrocyte autoantibodies[57, 58], it may also be caused by sulfasalazine-induced haemolysis in patients with glucose-6-phosphate dehydrogenase deficiency[56]. Although no clear correlation has yet been established between occurrence of AIHA and the degree of inflammatory activity in CU patients, a strong association has been demonstrated with the extent of bowel inflammation: Recent data show the incidence of AIHA in patients with extensive colitis to be as high as 28%[56]. AIHA, especially Coombs-negative AIHA, is rare in patients with CD, and only published as case reports[59, 60].

Anaemia in IBD patients can also occur in association with myelosuppression, whereby a number of different pathological mechanisms are to be considered. Data indicate a particularly high prevalence of myelodysplastic syndrome (MDS) in CD patients with colorectal disease, with MDS identified to be the determining prognostic factor[61]. While the

estimated prevalence of MDS in IBD patients is estimated at 17%, its annual incidence in IBD sufferers is approximately 170 per 100,000 patients, compared with 20-30 per 100,000 in the general population aged 70 and over[62]. MDS in IBD patients is assumed to be primarily drug-related (especially in thiopurine users, see below). However, the risk of MDS is evidentially increased in patients with autoimmune disorders in general, not least due to chromosomal anomalies of the bone marrow cells, which have been observed in 67% of IBD patients with concomitant MDS[63] and can trigger the production of colitogenic monocytes. In his hypothesis discussing the significance of microvascular injury in the pathogenesis of intestinal inflammation, Wakefield even suggested that Crohn's disease might represent an extrahaematological manifestation of MDS as an autoimmune reaction to vasculitis of the mesenteric arteries. Myelosuppression can also manifest as a complication of severe, extensive UC in the context of a systemic inflammatory response syndrome. Common pathogenic mechanisms and the increasing long-term use of immunosuppressants are additionally presumed to play a role. In particular, thiopurines, increasingly used for long-term maintenance therapy in IBD, are known to increase the risk for leukaemia and MDS. A large prospective French cohort study by Beaugerie et al. examined the prevalence of lymphoproliferative disorders in 19,486 IBD patients[64]. The relative risk of developing lymphoproliferative disease was found to be 5.2 times higher in those currently receiving long-term therapy with thiopurines compared to those who had never received thiopurine treatment.

2.9 Other gastrointestinal conditions

Last but not least, in IBD patients maintaining remission, the occurrence of IDA should prompt consideration of other GI conditions, such as gastrointestinal cancer or polyps and atrophic or H. pylori-associated gastritis (for review see[65]).

2.10 Inadequate dietary intake

An important, but often neglected, cause of micronutrient deficiencies resulting in anaemia is reduced dietary intake and avoidance of specific foods, especially during disease flares.

Several nutritional studies performed in different IBD cohorts reported inadequate dietary intake of vitamins and trace elements including iron, vitamin A, D, B_{12}, B_6, and FA, which, as already discussed, have been shown to be essential for sufficient haemoglobin synthesis[66-70].

3. **Anaemia workup**

Based on ECCO guidelines[4, 71], anaemia workup in IBD patients should be performed if haemoglobin is below normal, as defined by the WHO (Table 1). The European Crohn's and Colitis Organisation (ECCO) recommends that workup should include red blood cell indices such as red cell distribution width (RDW) and mean corpuscular volume (MCV), reticulocyte count, differential blood cell count, serum ferritin, transferrin saturation (TSAT) and C-reactive protein (CRP) concentration[4]. More extensive workup includes serum concentrations of vitamin B_{12}, folic acid, haptoglobin, percentage of hypochromic red cells (%HYPO), reticulocyte haemoglobin (CHr), lactate dehydrogenase, soluble transferrin receptor (sTfR), zinc protoporphyrin (ZPP), and creatinine (Table 3).

3.1 Diagnosing IDA and ACD

Because the interpretation of traditional biomarkers is frequently complicated by concomitant chronic diseases and polypharmacy, accurate diagnosis of IDA or ACD in IBD patients is often challenging. Although MCV and mean corpuscular haemoglobin (MCH) have been recommended as useful variables and are routinely determined in IBD patients within the complete blood count, MCV reduction is often lacking in the early stages of anaemia and/or blunted by other concomitant nutritional deficiencies (e.g. folic acid, vitamin B_{12}) or medications (e.g. azathioprine, methotrexate)[19].

In clinical practice, iron status is mainly assessed on the basis of serum ferritin levels, if at all. However, serum ferritin is not only subject to gender differences, but it is also an acute-phase reactant and thus prone to falsely elevated or normal levels in populations with inflammatory reactions (e.g. due to chronic conditions and hepcidin-mediated iron sequestration)[72]. Therefore, the diagnostic workup should include CRP and/or ESR, to

exclude underlying inflammatory reactions, and TSAT, a marker of low iron availability for haematopoiesis that is less affected by inflammatory reactions[19, 72]. Although TSAT is a more reliable marker of iron status, it is currently underused, being tested in only 25% of anaemic IBD patients[19].

Reticulocytes are those immature erythrocytes which still contain sufficient RNA to be microscopically detectable using specific alkaline stains. This blood count parameter (%) is obtained by flow cytometry and is available in all larger laboratories at reasonable cost. In chronic anaemia that has reached a steady state, relative reticulocyte count correlates inversely, though non-linearly, with the shortening of the erythrocyte lifespan. The absolute reticulocyte count, on the other hand, is a gauge for effective erythrocyte production by the bone marrow. Low or normal reticulocyte levels indicate inability to respond adequately to anaemia either because of inappropriate erythropoiesis caused by micronutrient deficiencies or due to primary bone marrow disease (=> hyporegenerative anaemia), whereas increased reticulocytes denote increased erythropoiesis (e.g. due to bleeding or haemolysis), thereby excluding micronutrient deficiencies (=> hyperregenerative anaemia). The reticulocyte production index (RPI) describes the relative reticulocyte count (RTC, in %) corrected to the severity of the anaemia (patient's haematocrit [HCT-P] in relation to ideal haematocrit [HCT-N = 0.45] and reticulocyte maturation time [RMT]): RPI = (RTC [%] x HCT-P)/(HCT-N [0.45] x RMT). The RPI thus represents the increase or decrease in erythrocyte production as a factor of the normal value[73].

An upregulation of the number of *soluble transferrin receptors (sTfR)* on cellular membranes continuously moving into the plasma reflects erythropoietic activity and inversely correlates with the amount of iron available for erythropoiesis[74]. Thus, sTfR was assumed to be useful marker of iron-deficient erythropoiesis resulting from absent iron stores in combination with restricted iron supply due to anaemia of chronic disease[75]. However, according to studies in rheumatoid arthritis and other inflammatory disorders, sTfR measurement is of limited value as a marker to determine the presence or absence of coexistent functional iron deficiency

(FID)[74, 76]. It may be hypothesised that a combination of haematologic markers such as reticulocyte haemoglobin content (see below), which decreases with iron deficiency, and TfR/F (sTfR-ferritin) ratio, may allow for a more precise classification of anaemia[77].

To date, only two studies have been published evaluating the accuracy of the TfR/F ratio in IBD patients[78, 79]. Applying a TfR/F ratio >1.4 as cutoff, Oustamanolakis et al. demonstrated a high discriminating power in the diagnosis of IDA, independent of CRP levels and/or disease activity (sensitivity 91%, specificity 92%)[78]. A considerably higher cutoff (TfR/F ratio >2) was used by Abitol et al., who most recently validated the TfR/F ratio as a useful biomarker for the diagnosis of true iron deficiency in their IBD patients[79]. Despite their promising results, both studies also showed that, because of its comparatively high costs and the lack of uniform cutoff levels (since reference ranges of the individual components are assay-dependent), TfR/F index cannot currently be recommended as a routine diagnostic tool, at least for primary anaemia screening. However, TfR/F index may be useful as an adjunct in the evaluation of anaemic patients whose ferritin values may be increased due to an acute-phase reaction[79]. In addition, it must be borne in mind that sTfR concentrations are increased in every expansion of erythropoiesis (i.e. haemolytic anaemia, thalassaemia or polycythaemia) and reduced in aplastic anaemia and other conditions with hypoproliferative erythropoiesis (e.g. renal anaemia).

Recent research has confirmed cytometry of the *reticulocyte haemoglobin content (CHr)* and the *percentage of hypochromic red cells* (%HYPO) to have a high predictive value in the differential diagnosis of IDA even when inflammation and ACD are present[80, 81]. While a reduction in %HYPO (mean lifetime, 120 days) denotes longer-term deficiency in iron supply, reduced CHr (mean lifetime, 48h) is an indicator of current iron deficiency, providing an accurate measurement of bioavailable iron over the previous 3–4 days. Also, CHr has been shown to be an early marker for treatment response following both oral and intravenous iron supplementation[82]. However, the test's utility is limited, as the percentage of hypochromic RBCs is dependent on the total number of RBCs, which may be affected by the length of

storage time. Therefore, the RBC samples cannot be shipped to offsite laboratories (Table 3).

Zinc protoporphyrin (ZPP) was pinpointed by Dagg and colleagues as early as 1966 as a potential indicator of ID[83]. A reduction in iron supply for erythropoiesis to a suboptimal level results in the production of ZPP instead of haem, with zinc, instead of iron, being incorporated into protoporphyrin IX. Thus, ZPP levels are a direct marker of iron status in the bone marrow during erythropoiesis. ZPP production is entirely unaffected by ACD or chronic inflammation and is therefore an effective indicator of ID even in the presence of inflammation. The onset of iron-deficient erythropoiesis triggers continuously increasing ZPP concentrations. Concentrations <40μmol/mol haem are considered normal. Values of 40–80μmol/mol haem represent latent iron deficiency (haemoglobin normal); >80μmol/mol haem are associated with manifest iron deficiency. In severe cases, values of up to 1000μmol/mol haem have been reported[84]. Measurement of ZPP concentration provides a reliable index of FID and may be used as an alternative to indices of red cell hypochromia or reticulocyte haemoglobin content, although it is less sensitive to acute changes in iron availability. If used in the assessment of FID, it is essential that measurements be made on washed RBCs, with the use of appropriate reference limits. Most recently, a study presented at the Digestive Disease Week (DDW) in 2014 demonstrated the diagnostic accuracy of zinc protoporphyrin/haem ratio for IDA screening in patients with IBD[85].

The utility of *hepcidin* measurement as a diagnostic tool is currently uncertain and for the time being this technique remains a research investigation. However, preliminary data of our own research group indicate that hepcidin level may be a powerful predictor of intestinal iron incorporation[86], which was most recently confirmed in a paediatric IBD population[87]. Thus, serum hepcidin can be considered a useful, sensitive surrogate marker to identify IBD patients who might benefit from oral iron supplementation, once accurate and feasible assays are available.

3.2 Diagnosis of vitamin B_{12} deficiency

Assessing for vitamin B_{12} status is definitively indicated in all patients with macrocytic anaemia or anaemia unresponsive to iron or erythropoietin. In addition, periodic screening should be considered in all CD patients, especially those with active ileal CD or history of ileal resection, although the recommended intervals for screening have not been established. ECCO guidelines recommend checking cobalamin levels at least annually or when macrocytosis is present, in the absence of thiopurine use[4].

Although there is no gold standard, diagnosis of vitamin B_{12} deficiency has traditionally been based on low serum vitamin B_{12} levels, usually below 200pg/mL (148pmol/L), along with clinical evidence of disease. However, a systematic review indicates that serum vitamin B_{12} levels alone are probably insufficient to diagnose deficiency in asymptomatic patients, and that such diagnosis requires verification by specific biomarkers[32]. Schilling's test is often used to elucidate whether malabsorption is present but does not help to quantify the effect this malabsorption has on body stores[32].

Therefore, the National Health and Nutrition Examination Survey (NHANES) guidelines are advocated, stipulating that the diagnosis of vitamin B_{12} deficiency in asymptomatic individuals should include one biomarker of circulating vitamin B_{12} (serum vitamin B_{12} or holotranscobalamin II) and one functional biomarker (methylmalonic acid; MMA or homocysteine; Hcy) to confirm intracellular vitamin B_{12} depletion[88].

In summary, initial anaemia workup must include red blood cell indices (MCH, MCV), reticulocyte count, serum ferritin, TSAT and CRP. In accordance with Figure 2, more extensive workup includes serum concentrations of vitamin B_{12} (HoloTC), folic acid, haptoglobin, %HYPO, CHr, LDH, sTfR, differential blood cell count, and creatinine.

4. **Treatment of anaemia**

Alongside adequate micronutrient supplementation (iron, vitamin B_6, B_{12}, D and folate), therapy should unquestionably focus on the identification and treatment of the underlying causes of anaemia.

According to ECCO guidelines, iron replacement in IBD patients should be initiated as soon as IDA is clearly ascertained or deemed likely due to ambiguous results for iron markers. In cases of iron deficiency without manifest anaemia, an individualised approach is required. The major goal of IDA therapy is to increase haemoglobin levels by >2g/dL or to normal values within 4 weeks, and to replenish iron stores, relieve anaemia-related symptoms and thereby improve quality of life[4].

Iron supplementation can be administered orally or intravenously. The latest ECCO guidelines state that intramuscular iron is now obsolete, since injections are painful, damaging to tissues and associated with unacceptable side effects[4]. The choice of route is determined by the symptoms, aetiology and severity of the condition, the dynamics of the haemoglobin decrease, comorbidities and individual risks of therapy.

4.1 Oral iron supplementation

Due to its convenience and low costs, oral iron supplementation is generally considered standard front-line therapy for iron-deficiency anaemia. Oral iron supplements are available as either ferrous (Fe^{2+}) or ferric (Fe^{3+}) salts. Because of the poor solubility of formulations containing ferric iron, the more widely-used forms are Fe^{2+} salts (for example, iron sulphate, gluconate or fumarate), assumed to have a similar efficacy and adverse effects profile in IBD, even though no head to head studies are available. However, a recent phase III trial demonstrated that ferric maltol is effective in IBD patients who are unresponsive or intolerant to ferrous sulphate[89].

The recommended daily dose is 100–200mg elementary iron for adults and 3–6mg/kg body weight (divided into two doses) for children[90]. However, its feasibility is compromised by poor absorption and gastrointestinal side effects including nausea, flatulence, diarrhoea and

gastric erosion[91], especially in the elderly IBD population[92]. After normalisation of haemoglobin levels, oral iron supplementation must be continued for at least 4 months to completely replenish iron stores, further decreasing the adherence rate, which has been estimated to be as low as 10%-32% even after only two months of treatment.
Moreover, both rodent models and human studies indicate that non-absorbed iron can cause potentially harmful modification of the gut microbiota, increasing the concentration of intestinal pathogens in both children and adults and thereby enhancing intestinal inflammation (for review see[93]) (Table 4).

In a most recently published trial, Moretti et al. confirmed that in women with depleted iron stores, iron absorption is highest at low iron doses (40-80mg). More importantly, the study demonstrated that low-dose iron given on alternate days may maximise fractional iron absorption, increase dosage efficacy, reduce gastrointestinal exposure to unabsorbed iron, and ultimately improve tolerance of iron supplements. Their findings emphasise the need to study longer-term, alternate-day schedules for iron supplementation in IBD patients[94].
Optimal timing for a switch from oral to intravenous (IV) iron replacement therapy is not well characterised in patient care or treatment algorithms. Based on a secondary data analysis of five randomised, controlled trials, Okam et al. recently reported that an Hb increase of 1.0g/dL or more at day 14 after starting oral supplementation may be the most accurate predictor of sustained treatment response: Sensitivity, specificity and positive predictive value of an Hb increase ≥1.0g/dL at day 14 were 90.1%, 79.3% and 92.9%, respectively[95].

4.2 Intravenous iron supplementation

Since the mean total iron dose generally required to correct anaemia and replenish iron stores in IBD patients is 1000–1500mg, ECCO guidelines recommend intravenous iron supplementation for the correction of IBD-associated IDA[4].

Intravenous iron has been demonstrated to be safe, effective and well tolerated in both the correction of IDA and the maintenance of iron stores in patients with IBD. Moreover, intravenous iron replacement not only facilitates the faster correction of ID and repletion of

body iron stores, but also effectively avoids GI side effects by bypassing the GI tract. Furthermore, ferritin levels following intravenous administration are higher than those achieved through oral iron intake, thus possibly reducing the likelihood of anaemia recurrence in the long term. Although intravenous iron is costlier than oral treatment, administration by a medical professional ensures compliance and more reliable repletion of iron stores, at least when single higher doses of iron are given[96].

Several intravenous iron preparations are currently available for treatment of IDA. Such formulations differ by complex chemistry and can be grouped into labile, semi-labile and stable iron complexes[97]. Large trials in IBD patients have been published for iron sucrose, iron dextran, ferric carboxymaltose and iron isomaltoside 1000, demonstrating efficacy and safety in terms of dosage (1000mg or ≤20mg/kg body weight) and therapy duration (for review see[24, 96, 98]).

Past experience of high molecular weight iron dextran (HMWID)-related anaphylactic reactions, in particular, has led to the general underutilisation of intravenous iron preparations. In recent years, safety has vastly improved owing to the development of new, well-tolerated intravenous (i.v.) iron formulations. A recent review issued by the US Food and Drug Administration (FDA) studying serious adverse reactions across the board in a range of i.v. preparations (LMWID, iron sucrose, ferric gluconate; but excluding HMWID) showed a cumulative rate of only <1:200,000[99]. These data were confirmed most recently by Wang et al. in a US Medicare nondialysis population[100]. In another study, the efficacy of HMWID and ferric carboxymaltose (FCM) were found to be similar, although FCM was associated with fewer hypersensitivity reactions. While current data are not sufficient to draw recommendations for a particular i.v. formulation, HMWID has now been removed from the market in the US and Europe. In 2013, the European Medicines Agency (EMA) published an assessment report[101] reviewing the risk of allergic reactions of all i.v. iron products registered in the European Union, which concluded that the benefits of i.v. iron-containing medicinal products continue to outweigh the risks in the treatment of ID situations when the oral route

is insufficient or poorly tolerated. Notably, in an effort to harmonise the Summary of Product Characteristics (SmPCs) of all i.v. iron products, the EMA removed the necessity of a test dose and imposed that staff trained to evaluate and manage anaphylactic or anaphylactoid reactions as well as resuscitation facilities should be immediately available when any i.v. iron product is administered.

Dosages for parenteral iron administration have traditionally been calculated using the Ganzoni formula (iron deficit [mg] = body weight [kg] x (target Hb - actual Hb [g/dL]) x 2.4 + 500 mg)[102]. However, the formula is not only error-prone and inconvenient; it is also inconsistently used in everyday clinical practice, and underestimates iron requirements. A simpler fixed-dose regimen (of ferric carboxymaltose) based on Hb and body weight was tested in IBD patients and found superior to the Ganzoni-calculated dosing (of iron sucrose) in terms of efficacy and compliance[103]. This novel dosing scheme may equally be utilised as a simple dosing guide for other iron formulations and patient groups. In cases of severe anaemia, it has been suggested to increase the iron dose by 500mg (Table 5). If high iron doses for efficient and speedy iron replenishment are required, ferric carboxymaltose and iron isomaltoside are the formulations of choice, since most clinical trial and observational data on high dose iron administration have been generated with these compounds.

Despite effective intravenous iron supplementation, anaemia recurs frequently and rapidly, reappearing in more than 50% of patients within 10-12 months[104]. In contrast to the traditional “watch and wait” strategy, the FERGImain and the PROCEEDextend trial both impressively demonstrated that the recurrence of IDA in IBD patients can be prevented[105, 106]. In addition, cost analysis favours such a proactive approach[4]. Therefore, haemoglobin indices and iron status should be monitored using Hb, ferritin, TSAT and CRP in IBD patients every 3 months for at least one year after correction, and every 6 to 12 months once Hb is normalised and iron stores are replenished. Figure 3 summarises the management of IDA in patients with IBD.

4.3 Treatment of vitamin B_{12} and folic acid deficiency

The optimal method for *vitamin B_{12} supplementation* in patients who have undergone ileal resection is unclear. The daily requirement of vitamin B_{12} is about 2.4μg[107]. In the case of existing vitamin B_{12} deficiency, parenteral (intramuscular or subcutaneous) administration remains the preferred route. Unfortunately, therapy recommendations regarding dosage and timing of vitamin B_{12} supplementation are largely inconsistent. However, the given dose must be calculated bearing in mind that clinical symptoms only begin to manifest when body vitamin B_{12} stores (4–5mg) are depleted to as little as 5%–10%[39]. The aim of therapy must be to fully compensate the deficit, and dosage should be chosen accordingly. Standard initial therapy for patients without neurological involvement is 1000μg i.m./s.c. three times a week for 2 weeks or daily for 5 days, followed by five further weekly injections of 1000μg. Maintenance treatment for patients presenting without neurological deficits is hydroxocobalamin 1000μg i.m./s.c. every 3 months. Hypersensitive reactions to hydroxocobalamin are relatively uncommon. Adverse reactions, which may be due to intolerance of cobalt or other constituents of the compound, can include nausea, flushing, pruritus, fever, dizziness, exanthema and (rarely) anaphylaxis. Occasionally, substitution therapy may be hampered by hydroxocobalamin-cyanocobalamin cross sensitivity[39]. Patients presenting with severe anaemia may develop transient hypokalaemia following B_{12} supplementation. While the clinical significance of this is unknown, potassium replacement therapy may be considered. In patients presenting with anaemia, a reticulocyte response should be evident after 7–10 days, provided the patient has adequate levels of iron and folate. If a haematological response is not achieved, the initial diagnosis should be reviewed. Suboptimal response may indicate previously masked iron and/or FA deficiency or the presence of another co-existing cause of anaemia[39].

Correction of megaloblastic anaemia may take up to 8 weeks. Existing neurologic dysfunction may transiently worsen and then subside over weeks to months[107]. Patients with ileal resection greater than 20cm must receive lifelong replacement[107]. A recent systematic review[108] suggest that vitamin B_{12}, given orally at a high dose of 1000–2000μg (initially daily,

then weekly, then monthly), is as effective as intramuscular injections in patients with B_{12} deficiency (1000µg B_{12}, delivering 10-30µg to the body). However, the studies did not include patients with CD, and it seems reasonable to assume that patients with IBD, especially active small bowel disease, may have impaired absorption of oral vitamin B_{12}. Therefore, further studies need to be performed before oral supplements can be widely recommended to IBD patients with B_{12} deficiency.

In *FA-deficient megaloblastic anaemia* due to dietary insufficiency or chronic medication (where vitamin B_{12} deficiency has been excluded), 5mg FA daily should be taken for 4 months, and up to 15mg daily for 4 months is suggested in malabsorptive states[39].
There are concerns that high intakes of FA supplements might mask the rare incidences of macrocytic anaemia associated with vitamin B_{12} deficiency by disguising an important diagnostic parameter[109]. Vitamin B_{12} status should thus be routinely evaluated in patients with FA deficiency before FA treatment is initiated.

4.4 Other vitamins

Evidence-based recommendations for effective *vitamin D substitution* in IBD are also currently lacking, but targeting serum 25-hydroxyvitamin D [25(OH)D] levels between 75 and 100nmol/L appears safe and may have also benefits for IBD disease activity[110]. Depending on baseline vitamin D serum concentration, ileal involvement in CD, body mass index and perhaps smoking status, daily vitamin D doses of 1800–10,000 IU/day are probably necessary[111]. Vitamin B_6 deficiency can be treated with 50–100mg/day of pyridoxine daily[112].

4.5 Erythropoiesis-stimulating agents and blood transfusion

In most IBD patients, treatment of the underlying disease in conjunction with iron and vitamin replacement is sufficient to effectively correct anaemia. In patients with ACD showing insufficient response to supplementation despite optimal IBD therapy and intravenous iron supplementation, treatment with erythropoiesis-stimulating agents (ESAs) and blood transfusion are further options. Several studies have underlined that the majority of these patients respond to ESA treatment with a significant increase in Hb levels and quality of

life[113]. A most recent systematic review shows that the addition of iron to ESAs improves haematopoietic response, reduces the necessity for RBC transfusions and improves Hb levels, and also appears to be well tolerated. To minimise adverse events (venous thrombosis and/or cardiovascular events), maximal target haemoglobin value should be limited to 12g/dL in patients with cancer or renal insufficiency.

Red blood cell (RBC) transfusion should only be considered when haemoglobin concentration is below 7g/dL, in the presence of particular risk factors such as severe comorbidities, or when faced with a life-threatening situation[4]. There is now growing evidence concerning post-operative mortality and morbidity following blood transfusion, showing even the transfusion of a single RBC unit to be associated with an adverse clinical outcome[114]. Consequently, a restrictive approach to RBC transfusion is warranted[115]. Since transfusions have no lasting effect and do not sufficiently replenish iron stores, other options (including i.v. iron with or without ESAs) should be considered[4].

5. Expert Commentary

Anaemia, both IDA and non-IDA, is now recognised to be an important complication in IBD patients with significant repercussions on quality of life and hospitalisation rates. However, weaknesses in its clinical management are still evident, with diagnosis and therapy lacking a standardised approach.

To further improve clinical management of IDA, better diagnostic tools are needed both for anaemia screening (e.g. zinc protoporphyrin) and as predictors of sustained therapeutic response (e.g. hepcidin, CHr). While the introduction of a new class of highly effective and safe intravenous iron formulations has substantially improved the repertoire of therapeutic options, there still some open issues concerning the prevention of relapsing IDA which should be the focus of future trials. In particular, studies are needed to address the long-term safety of high doses of IV iron and the utility of remission therapy to ensure and sustain normal haemoglobin and ferritin levels and to maintain patient quality of life.

Analogous to IDA, in the screening and therapeutic management of macrocytic anaemia, there is a need for more clinical data concerning the optimal use of biomarkers (Homocysteine, HoloTC, MMA), in order to enable standardisation of diagnostic procedures and therapy.

Last but not least, even though data regarding the role of other micronutrients (vitamin A, D, B6) are still scarce, clinicians should be aware of their role as possible causative factors for anaemia. The development of more convenient and accurate diagnostic markers and supplementation strategies should be addressed in future studies.

6. Five-year view

Anaemia is one of the most frequent extraintestinal manifestations of IBD. In recent years, major advances have been made in understanding the pathophysiology of ACD and IDA in IBD, allowing the development of new, improved diagnostic and therapeutic strategies. However, although anaemia is an important factor in the overall well-being of IBD patients, it is still frequently neglected, and routine diagnostic and therapeutic approaches are slow to adapt. Further studies are therefore needed to improve awareness of anaemia in physicians treating IBD patients, and to establish standardised treatments to inhibit the development and recurrence of anaemia and improve the clinical course of IBD. In the future, therapies focused on hepcidin are likely to further improve treatment options and outcome.

7. Key issues

■ Anaemia is the most common systemic complication and extraintestinal manifestation of inflammatory bowel disease.

■ Although there are several causes of anaemia in IBD, the two most frequent aetiologies by far are iron deficiency anaemia (IDA), and anaemia of chronic disease (ACD). In the majority of cases, however, IBD-associated anaemia is a prime example of combined IDA and ACD.

■ Common biochemical values are an inadequate basis for assessment of iron status of patients who have an inflammatory condition such as IBD.

■ While vitamin B_{12} and folate deficiency-associated and drug-induced anaemia are less widespread, these possibilities should also be considered.

■ Serum vitamin B12 levels alone are not adequate to diagnose deficiency in asymptomatic patients.

■ The major goal of therapy for IDA is to supply sufficient iron to increase haemoglobin levels by >2 g/dL or increase them to normal values within 4 weeks, and to fully replenish iron stores.

■ Iron supplementation should be administered intravenously to patients with IBD, even though many respond to oral iron.

■ In the light of post-operative mortality and morbidity following blood transfusion, its lack of long-term effect on Hb, and its inability to replenish iron stores, a restrictive approach to RBC transfusion is warranted.

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Declaration of Interest

J. Stein has received consultancy fees from AbbVie, Fresenius-Kabi, Immundiagnostik, MSD, Pharmacosmos, Takeda, and Vifor. Dr. Stein has also received payment for lectures from Abbvie, Falk Foundation, Ferring, Immundiagnostik, MSD, Pharmacosmos, Takeda, Thermofischer and Vifor. Additionally, Dr. Stein has received payment for manuscript preparation from Abbvie, Falk Foundation and MSD. A. U. Dignass has received consultancy fees from Abbott, MSD, Ferring, UCB, Otsuka, Roche/Genentech, Takeda, Pharmacosmos, Holystone Biotech and Falk Foundation. Dr. Dignass has also received grants from Institut für Gemeinwohl and Stiftung Leben mit Krebs as well as payment for lectures including service on speakers' bureaus from Falk Foundation, Ferring, MSD, Abbott, Otsuka, Vifor, Stiftung Leben mit Krebs, Kompetenznetz CED, Takeda and Pharmacosmos. Additionally, A. Dignass has received payment for manuscript preparation from Falk Foundation and payment for development of education presentations from Abbott, Pharmacosmos, Falk Foundation and Ferring. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Figure legends:

Figure 1. The pathogenesis of ACD is based on the combined action of hepatocyte- and macrophage-derived hepcidin and inflammatory cytokines:

(A) cytokine (IL-1, IL-6)-stimulated hepcidin induces ferroportin degradation, leading to impaired intestinal iron absorption and iron release from RES, trapping iron in macrophages, hepatocytes and enterocytes resulting in *iron restricted erythropoiesis; (B)* reduced renal erythropoietin release (EPO) resulting in decreased erythropoiesis; *(C)* direct suppression of erythropoiesis by cytokines (TNF, IL-1, IL-6, and IFN-γ) in the bone marrow, exerting pro-apoptotic effects on the earliest erythroid-committed precursor BFU-e (burst-forming unit-erythrocyte) and also as a result of impaired EPO production due to direct inhibition by the EPO promoter; (*D)* enhanced erythrophagocytosis by cytokine (TNF, IL-1, IL-6, IFN-γ) - activated macrophages, leading to *shortened erythrocyte lifespan*. Moreover, cytokines interfere with the EPO signaling process and downregulate EPO receptors, thus inducing resulting in *"EPO resistance"*[31]. Reproduced with permission from the Canadian Medical Association Journal (ISSN: 08203946), vol. 179, no. 4, 2008©. Original article by Zarychanski, R and Houston, D.S. This work is protected by copyright and the making of this copy was with the permission of Access Copyright. Any alteration of its content or further copying in any form whatsoever is strictly prohibited unless otherwise permitted by law.

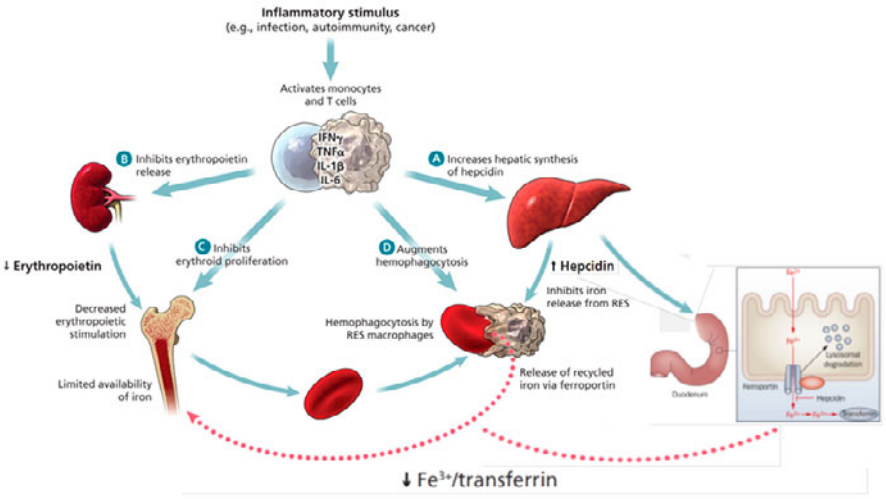
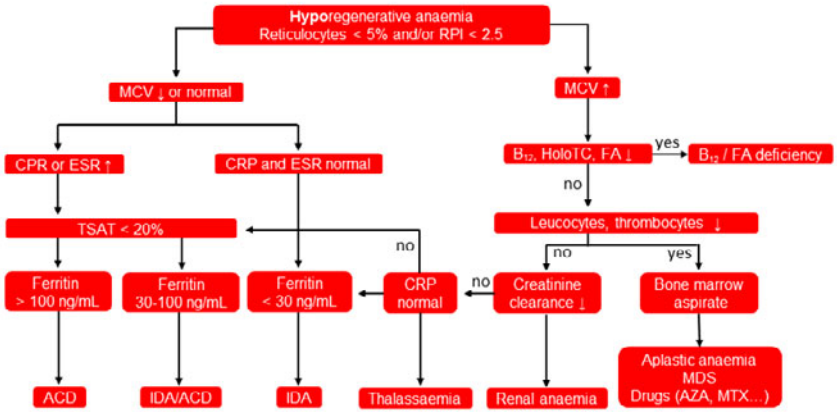


Fig. 1

Figure 2. Reticulocyte-based diagnosis of IBD-related anaemia: Anaemia can be extensively classified by combining reticulocytes, reticulocyte production index (RPI) and MCV: Low or normal reticulocyte levels indicate inability to respond adequately to anaemia either because of inappropriate erythropoiesis caused by micronutrient deficiencies or due to primary bone marrow disease (a; hyporegenerative anaemia), whereas increased reticulocytes denote increased erythropoiesis (e.g. due to bleeding or haemolysis), thereby excluding micronutrient deficiencies (b; hyperregenerative anaemia).

2a



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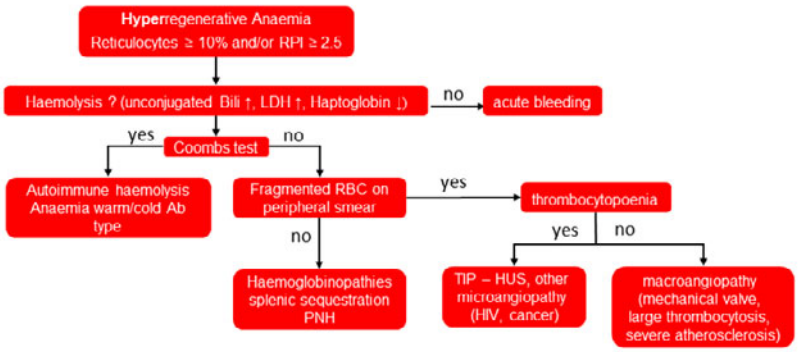
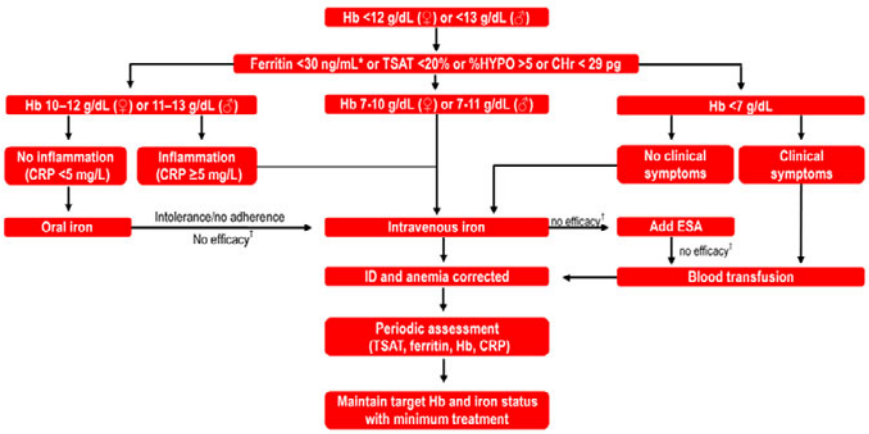


Fig. 2b

Figure 3. Workup for the management of iron deficiency anaemia in patients with IBD (adapted from[19,65])



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Reference annotations

** Of interest*

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Table captions:

Table 1: Minimum haemoglobin and haematocrit levels used to define anaemia in people living at sea level (WHO). Reproduced from WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.1). (http://www.who.int/vmnis/indicators/haemoglobin.pdf, accessed Oct. 2016) [5].

	Healthy	**Mild anaemia**	**Moderate anaemia**	**Severe anaemia**
Boys and girls (0·5–4 years)	**≥11.0**	10.0–10.9	7.0–9.9	<7.0
Boys and girls (5–11 years)	**≥11.5**	11.0–11.4	8.0–10.9	<8.0
Boys and girls (12–14 years)	**≥12.0**	11.0–11.9	8.0–10.9	<8.0
Non-pregnant women and girls (≥15 years)	**≥12.0**	11.0–11.9	8.0–10.9	<8.0
Pregnant women and girls (≥15 years)	**≥11.0**	10.0–10.9	7.0–9.9	<7.0
Men and boys (≥15 years)	**≥13.0**	11.0–12.9	8.0–10.9	<8.0

Table 2: Causes of IBD-related anaemia. Reproduced from Current treatment options in gastroenterology. Anaemia in the elderly IBD patient. 13, 2015, 303-318. Stein, J and Dignass, A.U. "with permission of Springer" [13].

Common	Iron deficiency anaemia Anaemia of chronic disease
Occasional	Cobalamin deficiency Folate deficiency Drug-induced [sulphasalazine, 5-ASA, 6-MP, azathioprine]
Exceptional	Autoimmune haemolysis Myelodysplastic syndrome Aplastic anaemia Glucose-6-phosphate dehydrogenase deficiency
Probable	Vitamin D deficiency Vitamin A deficiency Vitamin B_6 deficiency Copper deficiency

5-ASA, 5-aminosalicylic acid; 6-MP, 6-mercaptopurine

Table 3: Laboratory findings for the evaluation of anaemia in IBD [112, 116].

Parameter	Reference values*	Interpretation	Comment
MCV and MCH	MCV: 75–90 fl MCH: 27–33 pg per cell	Low levels can indicate concomitant true iron deficiency in ACD. Normal values do not exclude ID as up to 40% of 'pure' IDA cases are normocytic (e.g. in patients treated with AZA or 6-MP)	May be useful to guide iron repletion therapy; in some studies, less sensitive than TfR/ferritin ratio to indicate IDA
Ferritin	♀ 10–250 ng/mL ♂ 18–360 ng/mL	Low (<30 ng/mL): indicative of true iron deficiency even in the setting of inflammation. Normal/high (>100 ng/mL): inadequate iron stores in the setting of inflammation (CRP >5)	Ferritin expression is influenced by inflammation. True iron deficiency can also be present with higher ferritin levels (30 – 100 ng/mL)
Transferrin saturation (TSAT)	20–45%	Low: in ACD and ACD–IDA. High: acute or chronic iron overload (haemolysis, haemochromatosis)	Diurnal variation based on changes in serum iron concentrations. May be helpful for diagnosis of functional ID in the presence of high ferritin levels
Soluble transferrin receptor (sTfR)	0.8–3.3 mg/L*	High expression levels indicate iron requirements for erythropoiesis in the absence of inflammation	Sensitive to iron requirements for erythropoiesis, but expression is also suppressed by inflammation
Transferrin/ferritin ratio (TfR/F ratio)	N/A	>2: indicative of true iron deficiency in ACD <1: suggests functional iron deficiency	Better differentiation between ACD and ACD–IDA than sTfR alone, however, some overlap exits
Reticulocyte haemoglobin content (CHr)	28–35 pg	Reduced in ACD–IDA as compared with ACD; indicator for ongoing erythropoiesis and iron availability for reticulocytes	Determination dependent on specific technical equipment Overlap between ACD and ACD/IDA reduces discriminative potential
Hypochromic red blood cells (%HYPO)	< 5(6)%	Higher percentage in true iron deficiency; indicator for iron availability for erythropoiesis	Determination dependent on specific technical equipment Sensitivity for IDA in comparison to other

			methods unclear
Zinc protoporphyrin (ZPP)	< 40 µmol/mol Hb	40–80 µmol/mol Hb: ID without anaemia > 80 µmol/mol Hb: IDA	Should be interpreted cautiously in the setting of zinc deficiency. Not suitable to guide iron repletion therapy
Hepcidin	N/A	High levels in ACD Normal or reduced concentrations in ACD–IDA	Hepcidin levels seem to be more stringently controlled by iron requirements for erythropoiesis than by inflammation; assays not yet widely available
Haptoglobin (HPT)	300–2,000 mg/L	Reduced levels are indicative of haemolysis Increased levels may also be found in association with inflammation	Identification of haemolytic anaemia
Folic acid	2.0–9.0 ng/mL (4.5–20.4 nmol/L)	Decreases over time with ongoing erythropoiesis or gastric inflammation, or in association with treatment (e.g., methotrexate)	
Vitamin B_{12}	200–900 pg/mL (~147–645 pmol/L)	For clinical deficiency, sensitivity 95%–97% and specificity ≤ 80% For isolated biochemical deficiency, insufficient sensitivity and specificity (see table 4)	Should be part of initial and follow-up evaluation of anaemic patients with CD and ileoanal pouch
Vitamin D	25 OH vitamin D > 20 ng/mL:	< 20 ng/ml: deficiency 20-30 ng/mL: insufficiency > 30 ng/mL: sufficiency	1,25 OH vitamin D may be helpful for interpretation of HPT in the presence of normal calcium and 25 OH vitamin D levels

ACD, Anaemia of chronic disease; AZA; azathioprine; CD, Crohn's disease; CRP, C-reactive protein; Hb, haemoglobin; HPT, haptoglobin; ID, iron deficiency; IDA, iron deficiency anaemia; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; 6-MP, 6-mercaptopurine;

Table 4: Oral vs intravenous iron. Reproduced from [117].

	Oral Iron	**Intravenous Iron**
Pros	• Available over the counter • Convenient and inexpensive • Effective when intestinal absorption is not impaired	• Fast repletion of iron stores • Safe if formulations with dextran are avoided • Effective even in presence of inflammation
Cons	• Limited daily intestinal absorption results in slower Hb increase • ineffective repletion of iron stores • Dose-dependent gastrointestinal side effects (nausea, vomiting, abdominal pain, constipation) limits patient adherence. • Uptake is impaired in the setting of disease (eg, coeliac disease, anaemia of chronic disease, autoimmune gastritis). • Mucosal injury and/or potential exacerbation of disease activity may occur in inflammatory bowel disease. • Alteration of microbiota	• Requires administration by a health care professional, with associated increased costs • Potential for anaphylactic reactions with dextran-containing formulations

Table 5: Simplified scheme for estimation of total iron requirements. Reproduced from Gastroenterology, 141, Evstatiev, R. Marteau, P. and Iqbal T., *et al*. FERGIcor, a randomized controlled trial on ferric carboxymaltose for iron deficiency anemia in inflammatory bowel disease, 846-853, copyright (2011) with permission from Elsevier [103].

Degree of iron deficiency	Haemoglobin level (g/dL)	Dose (mg)	
		Body Weight <70 kg,	Body Weight ≥70 kg
No anaemia	Normal	500	1000
Moderate	10-12 (women) 10-13 (men)	1000	1500
Severe	7-10	1500	2000
Critical	<7	2000	2500