Commentary

Inflammation and iron homeostasis - what do blood tests mean?

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Iron is essential for humans and most microorganisms. It is needed to synthesize heme and iron-sulfur clusters, which are key components of many enzymes and proteins involved in vital cellular processes, like oxygen delivery, cytochrome function, ATP, and DNA synthesis[1]. In humans, regulation of iron homeostasis is crucial since both iron overload and iron deficiency have detrimental effects. Hepcidin is the master regulator of iron homeostasis, mainly produced by hepatocytes[2]. Its active isoform is a 25-amino acid peptide with structural similarities with defensins, a family of antimicrobial peptides of innate immunity. Hepcidin binds and inactivates ferroportin, the ubiquitous cell membrane iron exporter in mammalian cells[2], particularly expressed in spleen macrophages and enterocytes. This hepcidin-mediated regulation finely tunes macrophage iron recycling from senescent erythrocytes and iron absorption from duodenal enterocytes, perfectly balancing the iron need of erythroid bone marrow precursors and body iron losses. Hepcidin transcription is classically regulated by iron through an endocrine feedback loop, but many other negative and positive modulators can play a role[3]. IL-6 is one of the most powerful positive regulators[4]. During an infection, this ancestral mechanism leads to iron sequestration into macrophages to deprive of iron the pathogens as a part of a wider antimicrobial strategy known as "nutritional immunity"[5]. The resulting iron-restricted erythropoiesis is a major determinant of the so-called anemia of inflammation[6], which includes the category formerly known as anemia of chronic disorders (ACD), characterized by functional iron deficiency (FID). In FID, serum iron and transferrin saturation are typically low, while ferritin is increased. **Figure 1** shows the complex interplay between inflammation and iron homeostasis and the resulting alterations of the blood tests. Ferritin is a ubiquitous intracellular protein highly expressed by macrophages and hepatocytes, where it essentially serves to store safely iron. Its circulating concentration is very low (µg/L, as compared to transferrin, which circulates in g/L). In healthy individuals, serum ferritin reflects the amount of iron deposits, with every µg/L of ferritin corresponding to approximately 8 mg of stored iron[7]. Transferrin is an abundant extracellular protein deputed to safely transport ferric iron to various organs and tissues. While low ferritin levels (<20-30 µg/L) are virtually diagnostic for absolute iron deficiency (AID), high ferritin levels (>200-300 µg/L) are associated with iron overload only in a minority of cases. Ferritin is an acute phase reactant, but little is known about the physiological meaning of its secretion during inflammation, which can be massive during hemophagocytic syndromes. Recently, it has been shown that the H subunit of ferritin can stimulate macrophages to produce pro-inflammatory cytokines

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Blood Transfus. 2025; 23(1): 19-22 doi: 10.2450/BloodTransfus.967

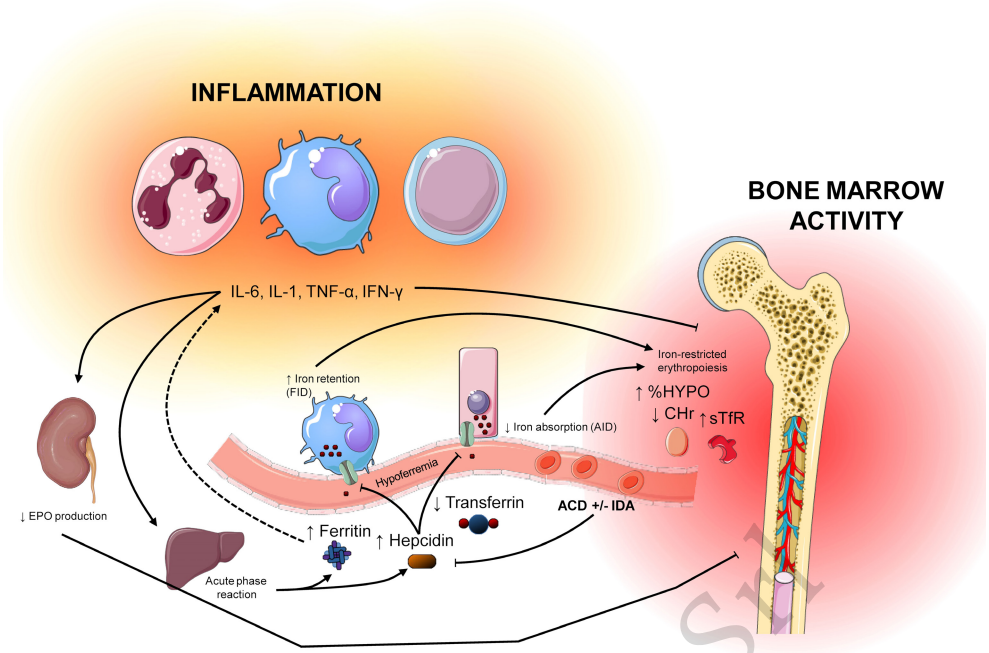

Figure 1 - Schematic overwiew of the complex interplay between inflammation and iron homeostasis, resulting in ACD ± IDA and perturbation of the biomarkers of iron status

%HYPO: percentage of hypochromic red cells; ACD: anemia of chronic disease; AID: absolute iron deficiency; CHr: reticulocyte hemoglobin content; EPO: erythropoietin; FID: functional iron deficiency; IDA: iron deficiency anemia; IFN-γ: interferon-γ; IL-1: interleukin-1; IL-6: interleukin-6; sTfR: soluble transferrin receptor; TNF-α: tumor necrosis factor- α.

and inflammasome activity, potentially perpetuating the vicious circle of inflammation[8]. Serum iron levels reflect the amount of transferrin-bound circulating iron. Normal levels are between 50 and 150 µg/dL, but they are poorly sensitive because of fluctuations due to circadian changes and diet.

An accurate evaluation of iron status in inflammatory diseases is important to either establish iron deficiency or avoid misdiagnosis of iron overload. Regarding the first point, this would imply solving the longstanding dilemma about iron supplementation in such frequent clinical conditions. Though population studies have proposed ferritin thresholds adjusted for the degree of inflammation, they are poorly reproducible[9]. The World Health Organization WHO has identified this point as a research gap[10], with particular implications for nutritional programs in developing countries where infections are widespread. In clinical practice, higher ferritin cut-offs have been proposed in patients with different chronic inflammatory disorders and in elderly subjects without overt comorbidities (e.g. with "inflammaging")[11] to diagnose AID and FID and pragmatically guide iron supplementation (**Table I**). The evaluation of TSAT, which derives from the ratio serum iron/(transferrin×1,42), is of paramount importance in these cases since it reflects the amount of biologically available iron. In absolute ID, transferrin is increased, and serum iron is reduced. Therefore, TSAT is low (<20%). Serum iron is reduced during inflammation, but transferrin can also be reduced since it behaves as a negative acute phase reactant. Therefore, the reduction of TSAT can be less pronounced. In this setting, other laboratory tests have been proposed to identify iron-restricted erythropoiesis better (**Table II**). Soluble transferrin receptor (sTfR) reflects the shedding of the membrane receptor, which is upregulated in case of low iron levels in erythroblasts. It appears to have higher sensitivity but lower specificity than ferritin in detecting ID. sTfR and sTfR/log ferritin (also named sTfR index or sTfR/ferritin index) showed improved detection of absolute ID in patients with ACD, being higher than in those with ACD without ID[12]. In a study of Skikne *et al.*[12], the sTfR and sTfR Index proposed

Table I - *Ferritin thresholds (ng/mL) for diagnosis of ID/indication to iron treatment according to the major international guidelines and recommendations*

Society	Ferritin threshold	Pts. category
British Society of Hematology (2021)	<15	Adult
British Society of Gastroenterology (2021)	<45	Adult
American Gastroenterology Association (2020)	<45	Adult
European Society of Cardiology and American Heart Association (2021 and 2022)	<100 <300 with TSAT <20%	CHF
European Society of Medical Oncology (2018)	<100	Cancer
British Columbia (2023)	<15-30	Adult
European Crohn's and Colitis Organization (ECCO) (2024)	<100	Active IBD
National Comprehensive Cancer Network (2020)	<500-800 (TSAT <50%)	Cancer
European Hematology Association (2024)	<30	Adult
Kidney Disease: Improving Global Outcomes (KDIGO)-National Kidney Foundation (2012 and 2025 draft)	≤500 (TSAT ≤30%)	CKD
International Consensus Conference on Anemia Management in Surgical Patients (ICCAMS) (2023)	<30 (and/or TSAT <20%) or <100 in inflammatory states	Perioperative anemia

CHF: chronic heart failure; CKD: chronic kidney disease; IBD: Inflammatory bowel disorders; TSAT: transferrin saturation.

Table II - *Summary of common and alternative/novel laboratory tests used to diagnose ID, and their variation during inflammation*

Laboratory tests	Physiologic meaning	Normal values	Variation during inflammation	Interpretation during inflammation
Ferritin	Intracellular protein which safely stores intracellular iron	30-300 µg/L	↑	Acute phase protein. Higher cut-offs are used to diagnose ID in inflammatory disorders
Serum iron	It reflects the amount of transferrin-bound circulating iron	50-150 µg/dL	↓	Hypoferremia is part of the "nutritional immunity"
Transferrin	Extracellular protein which safely transport ferric iron to various organs and tissues	2-4 g/L	↓	Negative acute phase reactant
TSAT	It reflects the amount of iron biologically available for erythropoiesis	20-45%	↓ / =	Less affected by inflammation
sTfR and sTfR/log ferritin	sTfR reflects bone marrow erythropoietic activity and iron status	Lack of standardization	sTfR is ↑ in co-existing ID sTfR/log ferritin is ↑ in ID and ↓ in ACD	sTfR/log ferritin may help detection of co-existing ID in patients with ACD
CHr and %HYPO	Early indicators of iron-restricted erythropoiesis	<28 pg and >5% suggest ID	Less influenced by inflammation	May help detection of ID in patients with chronic inflammatory disorders
25-Hepcidin	It regulates iron homeostasis by binding and inactivating ferroportin	Depends of age and sex, lack of standardization	↑ but depends also of concomitant disorders and treatments	May distinguish IDA from ACD in patients with chronic inflammatory disorders

%HYPO: percentage of hypochromic red cells; ACD: anemia of chronic disorders; CHr: reticulocyte hemoglobin content; ID: iron deficiency; IDA: iron deficiency anemia; sTfR: soluble transferrin receptor; TSAT: transferrin saturation.

cut-offs were 21 nmol/L (or 1.55 mg/L) and 14 nmol/L (or 1.03 using mg/L), respectively. Some Authors have also proposed sTfR to hepcidin or sTfR to ferritin ratios to improve the diagnosis of ID in the context of chronic heart failure[13]. However, sTfR assays lack standardization, and the sTfR indices have not gained sufficient popularity in clinical practice.

Flow cytometry cell analyzers can provide hemoglobin concentration and volumes of red blood cells and reticulocytes. They routinely analyze some cheap but

frequently overlooked additional parameters like reticulocyte hemoglobin content (CHr or Ret-He) and percentage of hypochromic red cells (%HYPO). Low CHr (<28 pg) is an early indicator of iron-restricted erythropoiesis, providing an indirect measure of the functional iron available for new red blood cell production over the previous 3-4 days[14]. It is also useful as an early measure of the response to iron therapy. Increased %HYPO (>5%) accurately detects insufficient bone marrow iron supply. It is particularly used to detect ID in CKD patients undergoing dialysis and treatment with erythropoiesis-stimulating agents (ESAs)[15].

Measurement of serum 25-hepcidin is a promising tool for assessing iron status during inflammatory disorders[3]. Inflammation is only one of the multiple opposing stimuli that can regulate circulating hepcidin levels. Positive regulators also include impaired renal clearance, iron administration, and repleted iron stores. Negative regulators are stressed erythropoiesis, hypoxia, ESAs administration, chronic liver disease, alcohol abuse, HCV infection, and, in particular, iron deficiency. Experimental and clinical evidence suggests that whenever inflammation and ID are both present, hepcidin suppression by ID tends to prevail[16]. Low hepcidin levels have been shown to distinguish IDA from ACD in patients with rheumatoid arthritis, cancer, and inflammatory bowel disease (IBD)[17]. In patients with CHF, hepcidin levels tend to decrease in advanced stages, reflecting multifactorial stimuli[18]. Some pilot studies found that hepcidin levels can predict response to i.v. iron[19]. However, the currently available serum hepcidin assays also lack full standardization, and, as for every hormone, the clinical interpretation of serum levels in any given patient requires a comprehensive evaluation[3].

In summary, there is an urgent need for further studies on the laboratory evaluation of iron status during inflammation, with a particular focus on iron deficiency. This is driven by global epidemiologic changes, with populations increasingly composed of elderly and patients with inflammatory multimorbidity. In the near future, robust revisitation of ferritin thresholds and a more widespread use of novel parameters will hopefully help clinicians face this common problem in daily practice.

The Authors declare no conflicts of interest.

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