Review Article

Exploration of iron metabolism: what is new?

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Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Abstract: Many biological analyzes or imaging techniques are currently available to explore iron metabolism and supply. In most cases, the first-line dosage of ferritin is recommended, especially in the diagnosis of martial deficiency, although this marker underestimates iron deficiency in case of inflammation. In patients with comorbidities, the exploration of iron metabolism becomes more complex and requires other tools. For example, the concentration of soluble transferrin receptors is insensitive to inflammation, thus affirming iron deficiency in patients with inflammatory syndrome. In iron pathologies, the transferrin saturation coefficient (TSAT), involving the assay of serum iron and transferrin, may direct the etiology of iron overload. There are also non-invasive nuclear magnetic resonance and computed tomography techniques to assess liver iron overload. Therefore, the exploration of iron metabolism relies on various heterogeneous tools ranging from biomarkers to imaging, including genetics. They must be used in the proper context.

Keywords: Iron metabolism; iron deficiency; iron overloads; hemochromatosis; ferritin; transferrin

Received: 29 June 2021; Accepted: 30 September 2021; Published: 30 October 2021.
doi: 10.21037/jlpm-21-50
View this article at: https://dx.doi.org/10.21037/jlpm-21-50

Introduction

The exploration of iron metabolism is of interest in many fields and pathologies. Iron is essential for oxygen transport through the heme of hemoglobin contained in red blood cells, cellular respiration like electron transport chain, DNA synthesis, and many other metabolic pathways. Iron is involved in the severity of diseases such as heart failure. The numerous modifications of the parameters depending on the patient's condition lead to difficulties interpreting the iron balance. Exploration of iron metabolism is a routine test that most laboratories should be able to perform. For example, iron deficiency (ID) is the most common micronutrient deficiency worldwide, with >20% of women experiencing it during their reproductive lives (1). Most etiologies of ID occur in the digestive tract, in men and postmenopausal women, and justify a morphological examination of the gut (2). Its prevalence is high, especially in children, women of childbearing age, pregnant women, and chronic pathologies such as kidney failure or cancer (3). Authors have suggested the role of ID anemia in worsening the clinical course of patients (4-6). Iron is involved in neurodevelopment (7,8), recovery after surgery (9), and survival in intensive care unit (ICU) (10,11). Furthermore, anemia could coexist with inflammatory bowel disease (IBD) in up to two-thirds of patients whose primary etiology is ID (prevalence ranging from 36% to 76% of patients) (2).

The exploration of iron metabolism is also of interest in some genetic diseases such as hereditary hemochromatosis (12) or sideroblastic anemia (13).

Table 1 Reference values

Nature	Reference values (to be adapted according to the dosing method used)
Serum iron	Male 10–30 µmol/L
	Female 9–29 µmol/L
	Newborn 10–36 µmol/L
	2 months to 15 years 11–24 µmol/L
Transferrin	Adult 2–4 g/L
	Newborn 1.6–1.8 g/L
Ferritin	Male 20–300 µg/L
	Female 15–150 µg/L
	Newborn 50–400 µg/L
	6 months-15 years 15–80 µg/L
sTfR	0.76–1.76 mg/L
Hepcidin	1–20 ng/mL (LC-MS-MS)
NTBI or FI	0.3–1 µmol/L

Serum iron in µmol/L; transferrin in g/L; ferritin in µg/L; sTfR in mg/L; hepcidin in ng/mL; NTBI or FI in µmol/L. FI, free iron; LC-MS-MS, liquid chromatography tandem-mass spectrometry; NTBI, non-transferrin bound iron; sTfR, soluble transferrin receptor.

Investigations

Biomarkers

The better non-invasive laboratory tests for diagnosing ID are serum ferritin concentration and the transferrin saturation coefficient (TSAT). Other parameters can be used to interpret the iron balance, especially in multiple pathologies leading to difficulties in interpreting the basic parameters. The commonly used units are presented in *Table 1*.

Ferritin

Ferritin reflects the body's iron stores. Ferritin blood concentration is proportional to the total body iron stores (14). Its decrease is the hallmark of an ID (with or without anemia), unlike its increase, which is not necessarily the reflection of an iron overload (15).

Ferritin is usually quantified by immuno-enzymology, chemiluminescence, immunonephelometry, or immunoturbidimetry. Circulating isoferritins are rich in L subunits and strongly glycosylated.

Transferrin

Transferrin is the main transport protein of reduced iron in the body. The body's iron stores partly regulate the hepatic synthesis of transferrin through the iron-responsive element (IRE)/iron regulatory protein (IRP) system (16). The synthesis of transferrin is induced by estrogen (pregnancy, estrogen-progestogen).

Therefore, transferrin concentration is inversely proportional to the serum iron concentration. The synthesis of transferrin increases, and the saturation of transferrin with iron decreases when the body's iron stores are low. Conversely, the synthesis of transferrin decreases, and the saturation of transferrin with iron increases when the body's iron stores are high.

Immunochemical techniques assay for transferrin includes mainly immunoturbidimetry. The reference material, CMR 470, makes it possible to standardize the antibody of the assay due to the variable degree of sialylation of the molecule.

Hypertransferrinemia results from increased liver synthesis; it could be due to ID.

Hypotransferrinemia is found in malnutrition, severe liver damage by decreased synthesis, urinary loss (nephrotic syndrome), and inflammatory syndrome by hypercatabolism.

Atransferrinemia is a severe disease that manifests itself from birth as severe hypochromic anemia. The iron absorbed is not transported efficiently and accumulates in the organs (liver, kidneys, pancreas, and heart).

Total iron-binding capacity or TBIC measures the amount of iron that can be carried into the bloodstream by transferrin. TIBC (µmol/L) = transferrin (g/L) ×25, or, TIBC (mg/L) = transferrin (g/L) ×1.395.

TSAT

TSAT is calculated according to the following formula: (%) = serum iron (µmol/L)/TIBC (µmol/L).

TSAT and TBIC are usually used to diagnose hemochromatosis (TSAT >45%). TSAT will generally be under 20% in the case of ID.

Detecting iron circulating in the blood is not recommended in the current clinical practice, notably due to significant

nychtemal variations. However, the iron measurement is notably mandatory for TSAT calculation. Current techniques for determining serum iron are colorimetric. Automated methods include colorimetric and immune methods with various interferences (bilirubin, hemoglobin, drugs, copper, etc.). Increased iron concentrations are observed in iron overloads, both acquired and inherited and hepatic cytolysis. The decreases reflect ID and acute and chronic inflammatory conditions.

Soluble transferrin receptor (sTfR)

sTfR is a truncated monomer of the extracellular domain of the tissue receptor. The isoform lacks its first 100 amino acids, which circulate in the form of a transferrin complex and its receptor. Its plasma concentration is correlated with the number of TfRs, and therefore with the iron status. Tissue deficiency leads to an increase in sTfR. Iron deprivation leads to increased synthesis of sTfRs, while excess iron depletes them. The availability of iron will therefore modulate the density of receptors on proliferative cells (17). At birth, the RsTF concentration is more than double that of adults. Then, quickly, there is no longer any variation related to sex or age. On the other hand, black subjects and people living at altitude have increased by 10% RsTF concentration compared to expected.

RsTF can be assayed by enzyme-linked immunosorbent assay (ELISA), immunoturbidimetry, or by immunonephelometry. The reference values vary according to the techniques, the monoclonal antibodies, and the units used (mg/L or nmol/L).

Hepcidin

Hepcidin's effect on a cellular level involves binding ferroportin, the principal iron export protein. This binding results in its ferroportin internalization and degradation and lead to iron sequestration within ferroportin-expressing cells. Aberrantly increased hepcidin leads to systemic ID and/or iron restricted erythropoiesis (18). Inflammation and iron refractory iron deficiency anemia (IRIDA) are associated with high hepcidin concentrations (6).

Hepcidin can be assayed by mass spectrometry (LC-MS-MS) or enzyme immunoassay (ELISA), giving correlated but different values (19).

The current or potential indications for the hepcidin assay in clinical practice are as follows:

- Microcytic hypochromic anemia with a suspected hereditary origin;
- Hereditary hemochromatosis: for therapeutic monitoring;
- Chronic renal failure and chronic inflammatory syndrome: to assess the indication for iron supplementation.

Free protoporphyrin

Its assay is carried out after separation by high performance liquid chromatography (HPLC) by fluorometric detection. In ID, protoporphyrin is increased in the urine (>700 µg/L). However, it can also be grown in chronic conditions or cancer.

Zinc protoporphyrin (ZPP)

ZPPs are determined by the hematofluorometric method on whole blood. However, concentration increase is not specific to ID. Still, it reflects an anomaly in the metabolism of the heme: when the iron is in insufficient concentration, zinc is incorporated in the heme tetrapyrrole ring.

It can be modified in hemoglobinopathies, sideroblastic anemia, inflammation, and lead poisoning (20,21). This assay is cheap and easy; it can eliminate ID in low concentrations.

Non-transferrin bound iron (NTBI)

This fraction comprises iron bound to proteins other than transferrin. Iron is also found in hemoglobin molecules associated with haptoglobin or within heme molecules, notably attached to hemopexin. It is linked to low molecular weight molecules, hence its name. When NTBI, which represents the free iron pool, is present in plasma, it is rapidly taken up by the liver, which could participate in toxic cellular effects and worsen hepatic iron overload. The values are on the order of 1 mM. When this compartment increases following an increase in iron from the extracellular medium, it would be responsible for the appearance of free radicals and cell damage. This fraction of the martial pool is the most toxic because it is metabolically challenging to control. The toxicity of iron is now well established. It is exerted at several levels, sometimes promoting the proliferation of microorganisms and infections, sometimes creating or worsening oxidative stress through Fenton and Haber-Weiss reactions, and inducing lipid peroxidation.

Iron not bound to proteins, also called low molecular weight iron, is the fraction of completely free iron, or pool of intracellular labile iron (LIP), which is in principle only found in the intracellular compartment of iron transit.

No standard method exists at this time.

These explorations must be coupled with:

- A complete blood count to check for an abnormality of erythrocytes and hemoglobin;
- A measurement of c reactive protein to assess a possible inflammatory syndrome.

Genetic analysis in iron overload

An increase in TSAT and/or hyperferritinemia should first lead to the search for the p.Cys282Tyr mutation of the *HFE* gene:

- If the patient is homozygous for the p.Cys282Tyr variant, it is a type 1 hemochromatosis. Apart from an associated inflammatory pathology, the serum ferritin concentration is a good indicator of iron overload. However, hepatic puncture-biopsy and/or magnetic resonance imaging (MRI) are considered for serum ferritin levels >1,000 µg/L with a high aspartate aminotransferase (AST) level and/or hepatomegaly.
- If the patient is not homozygous for the p.Cys282Tyr variant, the TSAT is a decision element:
  - With a TSAT comprised between 45% and 65%, it is necessary to suggest a compound heterozygosity p.Cys282Tyr-p.His63Asp, a type 4 ferroportin syndrome (regular or low TSAT with ferritinemia >1,000 µg/L), and a dysmetabolic hepatosiderosis (metabolic syndrome and secondary iron overload).
  - With a TSAT of >65% in a young subject, it is necessary to look for a cause of juvenile hemochromatosis.

In the presence of hypochromic microcytic anemia with normal or increased iron circulating concentration, testing for rare mutations may be undertaken.

Non-biological explorations

Nuclear magnetic resonance and computed tomography techniques are non-invasive techniques for the evaluation of liver iron overload. The invasive procedure of bone marrow aspiration with Perl's staining has long been the gold standard for the diagnosis of ID (20). Nuclear magnetic resonance and computed tomography techniques are non-invasive techniques for the evaluation of liver iron overload. The invasive procedure of bone marrow aspiration with Perl's staining has long been the gold standard for the diagnosis of ID (20).

Liver biopsy, a very invasive procedure with numerous contraindications, makes it possible to determine the amount of iron per gram of dry tissue and can be used to diagnose rare genetic overloads of genetic origin.

Interferences

Chronic inflammatory diseases such as cancer (22), infection (23), rheumatoid arthritis, IBD (6,24) are associated with modifying the iron balance and lead to interpretation errors. The determination of the additional parameters allows a better understanding of the iron balance of these patients (2,25,26).

High circulating concentrations of ferritin are found in various rare inflammatory medical conditions like macrophage activation syndrome, adult-onset Still's disease, catastrophic antiphospholipid syndrome (27). Some drugs will disrupt iron metabolism, such as cardiovascular drugs (1). Those interferences are summarized in *Table 2*.

Conclusions

Among the many biomarkers existing to explore iron metabolism, ferritin and transferrin saturation are generally used and sufficient in first line. In more complicated cases, especially in cases of coexistence of inflammatory disease, ferritin and transferrin saturation are generally not sufficient and a combination of specific biomarkers is often necessary to explore, particularly when comorbidities interfering with iron metabolism.

Biomarker ratios are increasingly developed to support the diagnosis of ID. Genetic studies also make it possible to refine the diagnosis in cases of overload. These biological analyzes eliminate the need for invasive examinations to explore iron metabolism.

Table 2 Exploration of iron metabolism in different pathologies. The most frequent patterns are summarized below

Biomarkers	Iron deficiency	Inflammation	ID + inflammation	ID + immunodeficiency	Hemochromatosis
Ferritin	↘	↗	↗ or N	↗ or N	↗
Transferrin	↗	↘	↘ or N	↘ or N	
Serum iron	↘	↘	↘ or N	↘ or N	
TSAT	↘	N	↘ or N	↘ or N	↗
sTfR	↗	N	↗ or N	N or ↗	
TIBC	↗	↘	↘ or N	↘ or N	
Hepcidin	↘	↗	N or ↘	N or ↘	
sTfR/log(ferritin)	>2	<1	>2	>2	
sTfR/ferritin	↗	N	↗	↗	

Serum iron in μmol/L; transferrin in g/L; ferritin in μg/L; sTfR in mg/L; hepcidin in ng/mL; NTBI or FI in μmol/L. ID, iron deficiency; TIBC, total iron-binding capacity; TSAT, transferrin saturation coefficient; sTfR, soluble transferrin receptor.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by editorial office, The *Journal of Laboratory and Precision Medicine* for the series "Physiology and Pathology of Iron Metabolism". The article has undergone external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm-21-50). The series "Physiology and Pathology of Iron Metabolism" was commissioned by the editorial office without any funding or sponsorship. KP served as the Guest Editor of the series and serves as an unpaid editorial board member of *Journal of Laboratory and Precision Medicine* from July 2019 to June 2021. KP gets some fees from for the consulting and educational events for Vifor France. The authors have no other conflicts of interest to declare.

Ethical Statement: All authors are accountable for all aspects of this work in ensuring that questions related to the accuracy or integrity of any part of this work are appropriately investigated and resolved.

References

1. Percy L, Mansour D, Fraser I. Iron deficiency and iron deficiency anaemia in women. Best Pract Res Clin Obstet Gynaecol 2017;40:55-67.
2. Murawska N, Fabisiak A, Fichna J. Anemia of Chronic Disease and Iron Deficiency Anemia in Inflammatory Bowel Diseases: Pathophysiology, Diagnosis, and Treatment. Inflamm Bowel Dis 2016;22:1198-208.
3. Lopez A, Cacoub P, Macdougall IC, et al. Iron deficiency anaemia. Lancet 2016;387:907-16.
4. Van Aelst LNL, Abraham M, Sadoune M, et al. Iron status and inflammatory biomarkers in patients with acutely decompensated heart failure: early in-hospital phase and 30-day follow-up. Eur J Heart Fail 2017;19:1075-6.
5. Lasocki S, Asfar P, Jaber S, et al. Impact of treating iron deficiency, diagnosed according to hepcidin quantification, on outcomes after a prolonged ICU stay compared to standard care: a multicenter, randomized, single-blinded trial. Crit Care 2021;25:62.
6. Cappellini MD, Comin-Colet J, de Francisco A, et al.

Iron deficiency across chronic inflammatory conditions: International expert opinion on definition, diagnosis, and management. Am J Hematol 2017;92:1068-78.
7. Wang Y, Wu Y, Li T, et al. Iron Metabolism and Brain Development in Premature Infants. Front Physiol 2019;10:463.
8. Iglesias L, Canals J, Arija V. Effects of prenatal iron status on child neurodevelopment and behavior: A systematic review. Crit Rev Food Sci Nutr 2018;58:1604-14.
9. Gómez-Ramírez S, Bisbe E, Shander A, et al. Management of Perioperative Iron Deficiency Anemia. Acta Haematol 2019;142:21-9.
10. Tacke F, Nuraldeen R, Koch A, et al. Iron Parameters Determine the Prognosis of Critically Ill Patients. Crit Care Med 2016;44:1049-58.
11. Lasocki S, Lefebvre T, Mayeur C, et al. Iron deficiency diagnosed using hepcidin on critical care discharge is an independent risk factor for death and poor quality of life at one year: an observational prospective study on 1161 patients. Crit Care 2018;22:314.
12. Brissot P, Pietrangelo A, Adams PC, et al. Haemochromatosis. Nat Rev Dis Primers 2018;4:18016.
13. Puy H, Manceau H, Karim Z, et al. Rare microcytic anemias. Bull Acad Natl Med 2016;200:335-47.
14. Polin V, Coriat R, Perkins G, et al. Iron deficiency: from diagnosis to treatment. Dig Liver Dis 2013;45:803-9.
15. Sandnes M, Ulvik RJ, Vorland M, et al. Hyperferritinemia-A Clinical Overview. J Clin Med 2021;10:2008.
16. Rouault TA. The role of iron regulatory proteins in mammalian iron homeostasis and disease. Nat Chem Biol 2006;2:406-14.
17. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clin Chim Acta 2003;329:9-22.
18. Ginzburg YZ. Chapter Two - Hepcidin-ferroportin axis in health and disease. In: Vitamins and Hormones. Elsevier, 2019;110:17-45.
19. Delaby C, Vialaret J, Hirtz C, et al. Analytical comparison of ELISA and mass spectrometry for quantification of serum hepcidin in critically ill patients. Bioanalysis 2021;13:1029-35.
20. Camaschella C. New insights into iron deficiency and iron deficiency anemia. Blood Rev 2017;31:225-33.
21. Braun J. Erythrocyte zinc protoporphyrin. Kidney Int Suppl 1999;69:S57-60.
22. Luporsi E, Turpin A, Massard V, et al. Iron deficiency in patients with cancer: a prospective cross-sectional study. BMJ Support Palliat Care 2021. [Epub ahead of print].
23. Bahi GA, Bidie AP, Meite S, et al. Exploring the iron metabolism in multidrug resistant tuberculosis (MDR-TB) patients in treatment. Int J Bio Chem Sci. 2017;11:1039.
24. Peyrin-Biroulet L, Williet N, Cacoub P. Guidelines on the diagnosis and treatment of iron deficiency across indications: a systematic review. Am J Clin Nutr 2015;102:1585-94.
25. Muñoz M, García-Erce JA, Remacha ÁF. Disorders of iron metabolism. Part II: iron deficiency and iron overload. J Clin Pathol 2011;64:287-96.
26. Frosch AEP, Ayodo G, Odhiambo EO, et al. Iron Deficiency is Prevalent among HIV-Infected Kenyan Adults and is Better Measured by Soluble Transferrin Receptor than Ferritin. Am J Trop Med Hyg 2018;99:439-44.
27. Rosário C, Zandman-Goddard G, Meyron-Holtz EG, et al. The hyperferritinemic syndrome: macrophage activation syndrome, Still's disease, septic shock and catastrophic antiphospholipid syndrome. BMC Med 2013;11:185.

doi: 10.21037/jlpm-21-50
Cite this article as: Melicine S, Peoc'h K, Ducastel M. Exploration of iron metabolism: what is new? J Lab Precis Med 2021;6:30.

