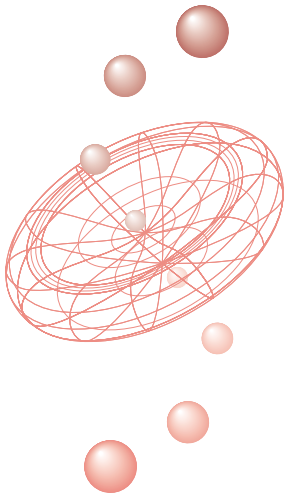
REVIEW ARTICLE



SERIES
13

The Analysis of Iron Status in Clinical Laboratories

Michael SCHAEFER and Torsten REINECKE

Sysmex Europe GmbH, Bornbarch 1,22848
Norderstedt, Germany.

Key Words

Iron Metabolism, Anemia, sTfR, CHr, RET-Y

INTRODUCTION

Worldwide iron deficiency is one of the most frequent causes for anaemia. Consequently, determination of iron stores is common for clinical laboratories. Recently, new methods and new test parameters have been developed allowing a wider and better analysis of the human iron status. A review of iron metabolism and the most frequent and valuable analytical parameters will be given.

IRON METABOLISM

A healthy individual absorbs approximately 1 mg of iron per day. This amount balances the natural daily loss of iron. ***Fig. 1*** illustrates the compartments of the body where iron metabolism occurs.
The loci for iron uptake are the duodenum and the small intestine. The amount of absorbed iron depends on the individual iron status. Patients with iron stores below normal values show increased iron absorption.
The iron transport is facilitated by ferritin, an iron binding protein. Ferritin moves iron from the iron stores (tissue transferring) to functional sites (in particular haemoglobin synthesis). That is why body iron may be categorized into three functional groups: functional iron, transport iron (in Transferrin) and storage iron. A simplified illustration of those three compartments of body iron is given in ***Fig. 2***.

HAEMOGLOBIN (HGB) CONTENT OF RED BLOOD CELLS (RBC) AND RETICULOCYTES (RET)

The HGB in mature RBC is measured as part of the complete blood count (CBC) as mean cell haemoglobin (MCH) content and as mean cell haemoglobin concentration (MCHC). MCH and MCHC as well as the mean corpuscular volume (MCV) are calculated from directly measured parameters as the RBC count, HGB concentration and the haematocrit (HCT). The red blood cell distribution width (RDW), an indicator of RBC anisocytosis, is obtained from the size distribution of cell volume around the MCV.
Some cell counters also measure the HGB content of RBC as the cellular haemoglobin concentration mean (CHCM) and the cellular haemoglobin content (CH). The distribution of directly measured cell haemoglobin concentration around the mean results in haemoglobin concentration distribution width (HDW), a measure sometimes used to characterize anisochromasia. To some extent such systems can also classify RBC-abnormalities as being hypochromic, normochromic, hyperchromic, microcytic, normocytic and macrocytic. In cases of suspected iron deficiency it is sometimes possible to determine the number of cells classified as hypochromic and/or microcytic. Equivalent parameters are also available for RET. The determination of the cellular HGB-content of RET is a direct insight into the iron status of the developing RBC, and yields information

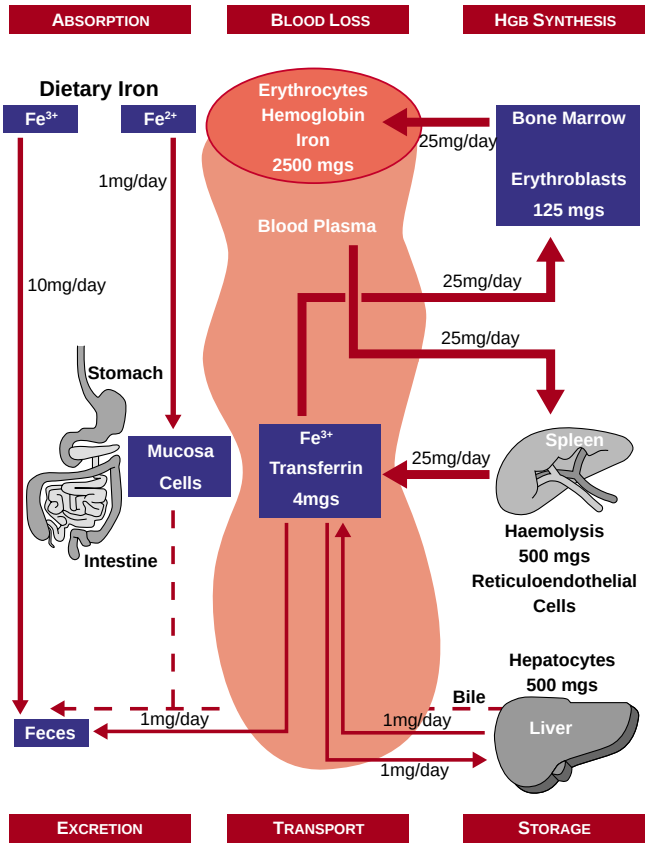


Fig. 1 *Balance of iron metabolism*[1)]

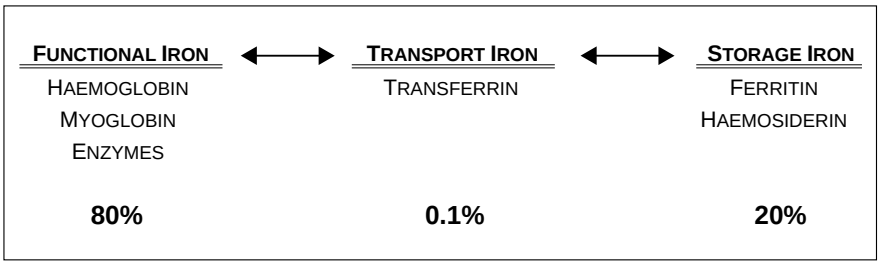


Fig. 2 *Iron compartments (in percent of total body iron)*

about the functional iron compartments of the body[2)].
A more recent and new haematology analyser, the Sysmex XE-2100, now provides highly valuable and interesting information in that regard. Such information is closer to the real cell biology since it uses fluorescence flow cytometry to individually analyse each RBC[3)].
Latest examinations with the XE-2100 revealed, that parameters obtained from the fluorescence flow cytometry channel have a better correlation with the "reference" (see below), the soluble transferrin receptor (sTfR), when compared to light-optical derived parameters like CHr[4)]*.

*Reference 4), the article is appeared in this journal (Vol. 11 No.2, PP 63-68).

Automated haematology analysers that accurately and reproducibly determine those values provide clinicians an easy and effective way to monitor changes in erythrocytic iron status. A patient undergoing iron therapy shows changes in those parameters after only 2 days. This is earlier than changes in white blood cell (WBC) count or changes in RBC count or CH.
There are additional studies that prove parameters like mean haemoglobin content of reticulocyte (CHr) or RET-Y are excellent indicators of "functional iron deficiency" (the "RET-Y" corresponds to the HGB-content of RET. It must however be stated, that this parameter is currently available as research parameter only). These respective parameters are obviously better indicators than serum ferritin, transferrin saturation or percentage of hypochromic cells, especially in patients with chronic renal failure. Also, monitoring erythropoietin (EPO)-therapy by controlling iron status can probably be successfully managed with such routine parameters.

THE SOLUBLE TRANSFERRIN RECEPTOR (sTfR)

RBC possess a trans-membrane protein called transferrin receptor. This protein can bind two molecules of transferrin. Transferrin which is fully saturated with iron binds to the receptor with higher affinity than partially saturated transferrin. The number of transferrin receptors depends on the patient's iron status: iron-deficient cells have more receptors than iron-depleted cells. Regulation of the receptor occurs at the mRNA level involving the iron responsive element binding protein (IRE-BP). Binding of IRE-BP to transferrin receptor-mRNA depends on the cellular iron pool[5].

sTfR was identified in serum, and serum levels were found to correlate with the number of cellular receptors. Today test-kits are commercially available to determine sTfR concentration quantitatively. The main clinical value of measuring sTfR is to help in the differentiation of functional iron deficiency from anaemia of chronic diseases (ACD). In functional iron deficiency the level of sTfR is increased while it remains at normal level in ACD. Other studies revealed that sTfR can predict which ACD patients having also developed iron deficiency. In these latter cases sTfR is increased but, not as markedly as in patients with simple iron deficiency[6].

sTfR has an advantage over the measurement of serum ferritin in that sTfR is not an acute phase protein. Therefore, sTfR can better discriminate between three conditions: iron deficiency, ACD and mixed iron deficiency and ACD (***Table 1***). Further refinements have been proposed by Punnonen, et al.[7], suggesting that the ratio sTfR/log ferritin (TfR-F index) is the best parameter to identify iron deficiency.

Levels of sTfR are influenced by changes in the erythropoietic rate. Where erythropoiesis is increased (*e.g.* autoimmune haemolytic anaemia, hereditary spherocytosis, polycythaemia rubra vera) the level of sTfR is increased. Conversely, in aplastic anaemia and chronic renal failure levels of sTfR are decreased.

REASONS FOR IRON DEFICIENCY

Impairment of iron balance can be due to increased demand, increased loss or reduced supply (***Table 2***). It is important not only to detect iron deficiency but also the cause of the deficiency.

Iron deficiency develops slowly in three stages: storage iron deficiency, iron deficient erythropoiesis and iron deficiency anaemia. Storage iron deficiency is characterised by reduced iron stores (in the storage compartment) without transport or functional compartments being affected. Iron deficient erythropoiesis arises when iron levels fall that much that the transport compartment becomes affected. Low transferrin saturation results in reduced iron transport to the developing RBC. Significant changes in erythropoiesis can be observed. However, HGB concentration remains within normal range limits. Iron deficiency anaemia is the final stage where lack of iron affects HGB production so that anaemia develops.

THE ANAEMIA OF CHRONIC DISEASE (ACD)

This condition is frequently associated with rheumatoid disease, malignancy and/or chronic infections. It is characterized by moderate anaemia that can be normochromic and normocytic or hypochromic and microcytic with changes in iron metabolism. Additionally, there may be a reduced RBC lifespan as well as reduced bone marrow response to anaemia. Low serum iron is commonly found despite the presence of normal iron stores. The reason is that iron from the reticuloendothelial system is not made available for erythropoiesis.

HYPOCHROMIC MICROCYTIC ANAEMIAS

The result of decreased production of one or more of the globin chains due to an inherited genetic abnormality is thalassaemia. There is a homozygous (severe) or heterozygous (mild) disease form. In ß-thalassaemia there is an underproduction of ß-globin resulting in lower red cell haemoglobin content with low MCV and MCH. With ß-thalassaemia trait, HbA_2 is usually raised while it is normal or low in α-thalassaemia. Patients with one of the thalassaemia syndromes can also present iron deficiency. Treatment of iron deficiency (increasing haemoglobin concentration while RBC remain microcytic and hypochromic) can be followed by thalassaemia investigation: Iron deficiency may cause a decrease in HbA_2 concentration. Therefore, it is important to know the patient's iron status when evaluating HbA_2 results.

Table 1 *Differentiation of hypochromic microcytic anaemiae*

	Iron deficiency	ACD	Mixed form
MCV	decreased	decreased	decreased
Serum iron	decreased	decreased	decreased
Transferrin saturation	decreased	decreased	decreased
Serum ferritin	decreased	normal-increased	normal-increased
sTfR	increased	normal	slightly increased
Bone marrow iron	decreased	normal-increased	normal-increased

Table 2 *Reasons for iron deficiency*

Decreased supply	Increased loss	Increased demand
Vegetarians	Acute bleeding	Childhood growth
Malnutrition	Chronic bleeding	Pregnancy
Malabsorption	Menorrhagia	
	Gastrointestinal bleeding	

Sideroblastic anaemia can be inherited (sex-linked or uncertain) or acquired (idiopathic or due to alcohol or drug abuse). The idiopathic type is a refractory anaemia with ringed sideroblasts present in the bone marrow. Characteristic for the peripheral blood picture is dimorphism of normochromic and hypochromic RBC. There can be prominent basophilic stippling. Bone marrow iron stores are usually increased and sometimes accompanied with leukopenia and/or thrombocytopenia. Sideroblastic anaemia can be considered as one of the myeloproliferative disorders[8].

IRON STATUS ANALYSIS IN CLINICAL LABORATORIES

A requirement for analysis and determination of iron status is the detection of the three phases of developing iron deficiency. Real iron deficiency is to be correctly differentiated from ACD. Using most recent analysis protocols in order to avoid unnecessary, laborious and expensive procedures can further increase laboratory efficiency.

The CBC is a basic test to detect the presence of anaemia and determine whether MCV, MCH, MCHC and RDW are within normal ranges. An increased platelet (PLT) count may indicate that the patient is bleeding. Blood smear examination can confirm the CBC and provides further RBC information as to rouleaux formation, polychromasia, target cells or basophilic stippling. At this stage, it is possible to suspect thalassaemia and consequently to request HbA_2 testing. HGB variants are to be checked by electrophoresis or HPLC.

The presence and degree of inflammatory diseases are detected and analysed by CRP (C-Reactive Protein) or ESR (Erythrocyte Sedimentation Rate) analysis. Modern haematology analysers (like the Sysmex XE-2100) also allow for the determination of immature granulocytes (IG) as a potentially high sensitive parameter and indicator for inflammatory conditions.

A further indicative parameter for the erythrocytic iron status is zinc protoporphyrin (ZPP). Iron depleted red cells show increased incorporation of zinc into their protoporphyrin: elevated levels of ZPP are detectable. Increased levels of ZPP are found in lead poisoning, as well. One advantage of determining ZPP is that it can be performed from the same sample used for the CBC.

Analyses of serum iron and transferrin have widely been replaced by serum ferritin. Transferrin saturation, however, is still recommended for verifying haemochromatosis. Patients with increased transferrin saturation can be selected to check for mutations of the HFE gene[9]. Serum ferritin is useful in less complicated cases of iron deficiency. It can also monitor iron overload. There are, however, serious limitations as soon as an inflammation is present.

sTfR has been found to be useful as it has become widely available nowadays. Many laboratories prefer to determine sTfR in addition to serum ferritin instead of serum ferritin only.

To access iron status prior to courses of EPO in chronic renal failure, the use of RET-Y or CHr is a fast and cost-effective measurement. Both parameters are also useful in monitoring response to iron treatment and to detect iron deficiency at an early stage. A study of iron deficiency in children revealed CHr as a stronger predictor when compared to sTfR[10]. This trend is confirmed by first studies at University College of London Hospital confirming RET-Y as being equivalent or even superior to CHr[4].

It has to be mentioned that bone marrow iron stores analysed via bone marrow smear staining is still the "gold standard" for the majority of haemotologists. Nevertheless, it should be thoroughly considered if patient's response to iron therapy is not a more practical and useful approach – with less pain for the patient concerned.

References

1) *Wick M, Pinggera W, Lehmann P: Iron metabolism. Anemias Diagnosis and Therapy, 15: 2000.*
2) *Fishbane S, et al.: Reticulocyte haemoglobin content in the evaluation of iron status of hemodialysis patients. Kidney International, 52: 217-222, 1997.*
3) *Inoue H: Overview of Automated hematology analyzer XE-2100. Sysmex J Int, 10: 58-64, 1999.*
4) *Briggs C, et al.: New red cell parameters on the Sysmex XE-2100 as potential markers of functional iron deficiency. Infus Ther Transfus Med, 28: 256-262, 2001.*
5) *Uchida T: Overview of iron metabolism. Int J Hematol, 62: 193-202, 1995.*
6) *Nielsen OJ, et al.: Serum transferrin receptor levels in anaemic patients with rheumatoid arthritis. Scand J Clin Lab Invest, 54: 75-82, 1994.*
7) *Punnonen K, Irjala K, A Rajamäki: Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood, 89: 1052-1057, 1997.*
8) *Bottomley SS: Sideroblastic anaemia. Clinics in Haematology, 11: 389-410, 1982.*
9) *Robson KJH: Diagnosis and management of haemochromatosis since the discovery of the HFE gene: a european experience. Br J Haematol, 108: 31-39, 2000.*
10) *Brugnara C, et al.: Effects of subcutaneous recombinant human erythropoietin in normal subjects: development of decreased reticulocyte haemoglobin content and iron-deficient erythropoiesis. J Lab Clin Med, 123: 660-667, 1994.*