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Review article

BIOCHEMICAL MARKERS AND HAEMATOLOGIC INDICES IN THE DIAGNOSIS OF IRON-RESTRICTED ERYTHROPOIESIS AND MONITORING OF r-HuEPO THERAPY

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Summary: Classical iron deficiency (ID) is not a challenge for the laboratory and physicians. The diagnosis is associated with microcytic hypochromic anemia and low serum ferritin. Anemia that accompanies infection, inflammation, and cancer, commonly termed anemia of chronic disorders (ACD) has apparently normal or increased iron stores. However, 20% of these patients have iron-restricted erythropoiesis (functional ID), an imbalance between iron requirements of the erythroid marrow and the actual iron supply. Functional ID leads to a reduction of red cell hemoglobinization, which causes hypochromic microcytic anemia. The early diagnosis of functional ID is based on the measurement of heamoglobin content of reticulocytes. This index can be estimated measuring the CHr with the Bayer Advia analyzer or the Ret-Y, using the Sysmex XE analyzer series. A CHr < 28 pg or a Ret-Y < 1630 are sensitive indicators of functional ID. For the assessment of iron status and the detection of advancing iron deficiency in patients with ID, ACD and the combined state of functional ID/ACD a diagnostic plot was developed. The plot indicates the correlation between the ratio sTfR/log ferritin (ferritin index), a marker of iron supply for erythropoiesis, and the CHr or Ret-Y. The diagnostic plot shows a good selectivity to assess the iron status of disease specific anemias like classical ID, endstage renal failure, cancer related anemia and anemia of infection and inflammation. Therapeutic implications of the diagnostic plot are to differentiate patients who should be administered with oral iron, with r-HuEPO or a combination of r-HuEPO and iron. The therapy can be monitored using the CHr, the Ret-Y, the sTfR or the diagnostic plot. An increase of CHr > 1 pg, of sTfR > 20% or an upward movement of the patient data point in the diagnostic plot indicates response to iron or r-HuEPO.

Key words: anaemia of chronic disorders, reticulocyte hemoglobin content, functional iron defiency, diagnostic plot

Introduction

Worldwide anaemia remains an enormous problem as more than 1 billion people suffer from this condition, the most important causes being iron deficiency, chronic inflammatory disorders and hereditary disorders of haemoglobin synthesis. Red cell production is a process regulated in the proliferation and the maturation pool of the bone marrow. In the proliferation pool the red cell progenitors require stimuli from cytokines for survival and proliferation. Every erythroid precursor cell needs at least stimulation of 10 erythropoietin molecules (1). A large quantitity of iron is needed in the maturation pool to make about 30 mL of red blood cells per day.

The pathogenesis of anaemia that accompanies infection, inflammation, and cancer, commonly termed anaemia of chronic disorders (ACD) (2) and the anaemia of endstage renal failure (3) are attributed to a blunted response of erythroid precursor cells to erythropoietin or a reduced synthesis of the cytokine (*Figure 1*). A reduced number of red cells arise from the marrow red cell precursor pool, however if enough iron is available, every precursor cell which enters the maturation pool will have a normal red cell haemoglobinization (4). In the complete blood cell count a mild normocytic normochromic anaemia is measured.

Patients with ACD present the following clinical laboratory results (*Table I*):

– Low serum iron and low transferrin saturation resulting from retention of iron by reticuloendothelial macrophages and reduced intestinal iron absorption.

– Normal ferritin concentration in their early course elevated values in the later stages because much iron is retained in macrophages.

– Reduced reticulocyte count because of hypoproliferative erythropoiesis.

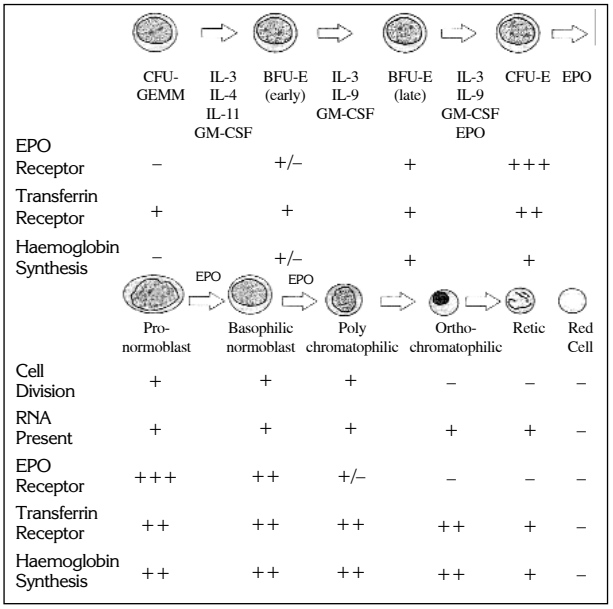

Figure 1. Development of erythropoiesis. With kind permission according to Ref (6). Red cell production progresses from the burst forming unit erythroid (BFU-E) to the colony forming unit erythroid (CFU-E) in the proliferation pool (top cell line). The main stimulating cytokine is erythropoietin. Maturation of the precursor cells to erythrocytes occurs in the maturation pool (bottom cell line). Maturation of red cells is mainly dependent on iron supply to erythropoiesis.

Table I Clinical laboratory results in anaemia of chronic disorders

Results	Clinical characteristics
Blood cell count – erythrocytes 3–4 mio/μL – Hb 90–120 g/L – MCV > 80 fl – MCH > 28 pg – % HYPO < 5%	Mild to moderate normocytic, normochromic anaemia
Serum – iron (Fe) < 7.16 μmol/L – ferritin > 20 μg/L – transferrin saturation < 20% – sTfR normal	Disturbances of iron distribution: – low serum iron – increase in iron stores – iron supply to the erythroid marrow is inadequate for the red cell precursor demand
Reticulocytes CRI < 0.8% or < 30 000/μL	Not appropriately increased for the degree of anaemia, indicating hypoproliferative erythropoiesis

Iron deficiency anaemia (IDA) results from a reduced iron supply for erythropoiesis in the maturation pool. Iron restricted erythropoiesis (IRE) can result from classical iron deficiency, the combined state of IRE with the ACD and in functional iron deficiency (*Figure 2*). The most important classical iron deficiency (ID) is not a challenge for the laboratory and physicians. The diagnosis is associated with microcytic hypochromic anaemia and low serum ferritin because of depleted body iron stores (4). About 20% of patients with ACD have iron-restricted erythropoiesis nevertheless they have repleted body iron stores (6). Anaemia results from impaired iron recycling. Functional iron deficiency (FID) is an imbalance between iron requirements of the erythroid marrow and the actual iron supply (7). FID is common in r-HuEPO treatment without iron supplementation (8). Functional ID like IRE leads to a reduction of red cell haemoglobinization, which causes hypochromic microcytic anaemia. The expressions IRE and FID are used as synonyms.

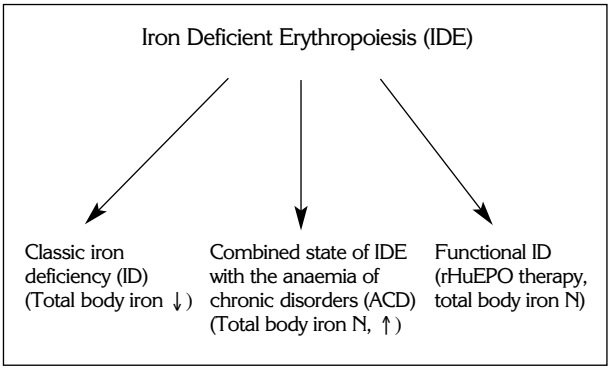

Figure 2. Iron-restricted erythropoiesis in classical iron deficiency, anaemia of chronic disorders and functional iron deficiency

Table II Biochemical markers and haematological indices for the evaluation of iron status

Marker	Assessment
Ferritin	Level of body's iron stores
sTfR	Iron demand of erythropoiesis
sTfR/log ferritin	Iron supply for erythropoiesis
CRP	Altered iron metabolism in ACD
HYPO (%)	Functional ID (time-averaged parameter)
CHr (pg)	Functional ID (acute parameter)
Ret-Y	Functional ID (acute parameter)

Laboratory tests for iron status include biochemical markers and haematological indices (*Table II*). The conventional biochemical markers include serum iron, transferrin/total iron-binding capacity, transferrin saturation and ferritin. Although widely used, these parameters are influenced by the acute phase response (APR) which complicates clinical interpretation of the test results. Ferritin is an acute phase reactant. The serum soluble transferrin receptor (sTfR) assay is yet another promising tool for the diagnosis of iron depletion (9). Calculation of the ratio sTfR/log ferritin (sTfR/log ferritin or sTfR-F index) is a way of combining sTfR and ferritin results (10). Besides the complete blood count new haematological indices which have gained merit in the assessment of iron status include the reticulocyte haemoglobin content measured as CHr or RET-Y (11, 12).

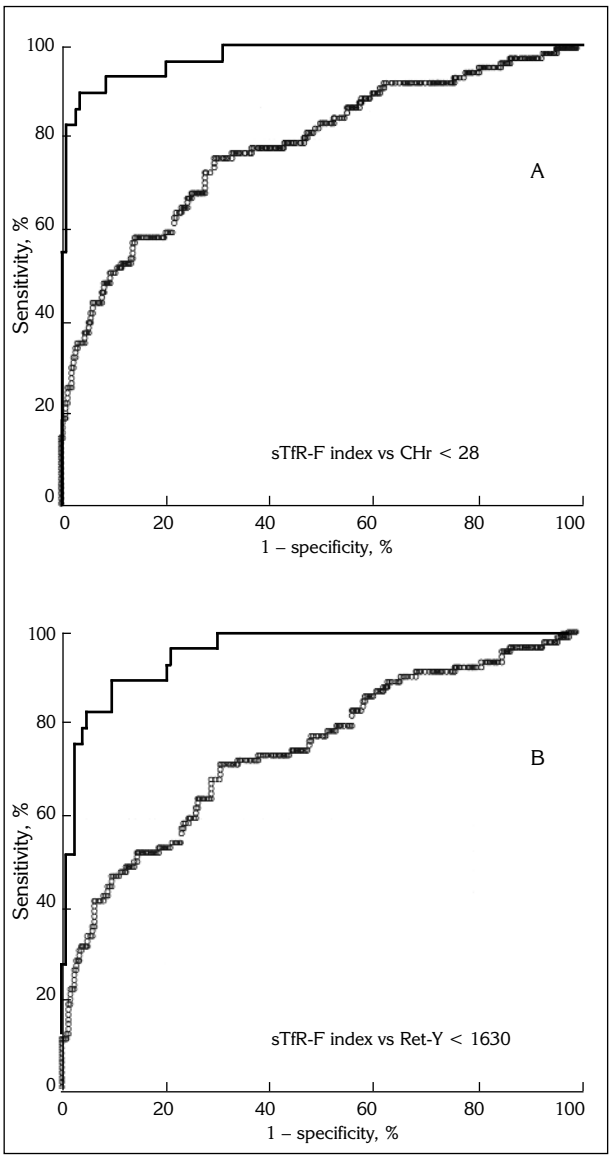

Figure 3. ROC plots showing the ability of sTfR-F index to indicate FID in patients with and without APR. As references CHr < 28 pg (top) and Ret-Y < 1630 AU (bottom) were used. The solid lines represent patients without APR (CRP < 5 mg/L), and the dotted lines represent patients with APR (CRP > 5 mg/L)

The objectives of our studies were threefold: (1) to compare the biochemical markers of iron status with the haematological indices for the detection of IRE, especially in patients with APR, (2) to develop a diagnostic plot combining haematological indices with biochemical markers as a tool for identification of different phases of advancing ID in anemic patients with and without APR, and (3) to evaluate the diagnostic plot for monitoring IRE in oral iron supplementation and FID in r-HuEPO treatment.

Comparison of biochemical markers of iron status with haematological indices for the detection of iron restricted erythropoiesis

Red cell haemoglobinisation, especially the haemoglobin content of reticulocytes provides direct evaluation of the bone marrow activity reflecting the balance between iron and erythropoiesis. Modern haematology analysers capable of identifying small subpopulations of erythrocytes within the total RBC population offer appropriate tools. The haemoglobin content of reticulocytes can be estimated measuring the CHr with the Bayer Advia analyzer or the Ret-Y, using the Sysmex XE analyzer series. We evaluated a reference range for CHr of 28–35 pg and for Ret-Y of 1630–1860 arbitrary units corresponding to a haemoglobin content (Ret-H_e) of 28.2–36.4 pg (6, 13).

The APR has a strong influence on the iron supply for erythropoiesis. Therefore the biochemical markers of iron status were compared with haematological indices in groups of anaemic patients with and without APR. The C-reactive protein (CRP) assay was used as a marker of inflammation, all patients with a CRP level greater than 5 mg/L being considered to have an inflammatory disorder. The sTfR level was measured using the Dade assay.

Using the CHr and Ret-Y < 28 pg as a gold standard for IRE the sTfR/log ferritin was the most accurate marker for biochemical identification of IRE. However, as shown from the area under curve from the ROC plots, the accuracy of the sTfR/log ferritin in patients with APR was insufficient (*Figure 3*). From these studies we found that patients with a sTfR/log ferritin higher than 1.5 have iron depleted stores while patients with an index less than 1.5 have stores which are iron replete. However, in patients with elevated CRP levels, the separation point is 0.8 since ferritin is an acute phase protein and is elevated in inflammato-

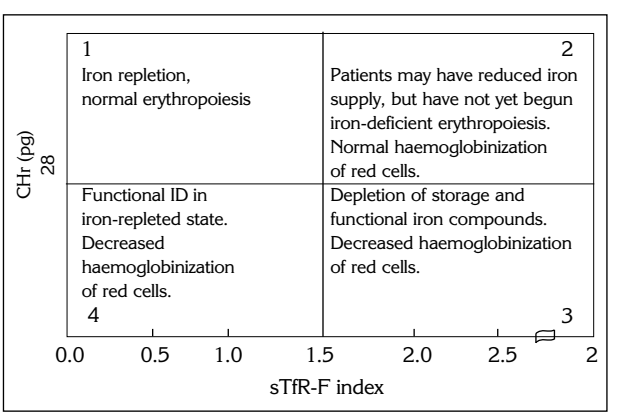

Figure 4. Diagnostic plot for identification of different phases of advancing iron deficiency combining the biochemically indicated iron supply (sTfR-F index) with the haematologically indicated iron demand (CHr) for erythropoiesis

ry disorders independently of the body iron stores. As a result the sTfR/log ferritin is increased and the decision point is moved to a ratio of 0.8 (6).

Development of a diagnostic plot for identification of different phases of advancing iron deficiency

The rationale behind the diagnostic plot was to combine the best marker of iron supply for erythropoiesis with the CHr or RET-Y (Ret-H_e) as indicators of bone marrow iron demand (*Figure 4*). Patients with a CHr level greater than 28 pg or RET-H_e greater than 28.2 pg (normal haemoglobinisation of reticulocytes) have not IRE or FID whereas those with CHr less than 28 pg or Ret-H_e less than 28.2 pg (reduced haemoglobinisation of reticulocytes) have IRE or FID. It is also important to know whether or not an inflammatory disorder coexists. For patients without inflammation, the ferritin index separating iron depleted from an iron replete state is 1.5 as previously stated. On this basis the diagnostic plot is divided into four quadrants. Patient data points in quadrant 1 indicate replete iron stores and normal red cell hemoglobinisation. In this quadrant are located patients with cancer-related anaemia (CRA), ACD, and end stage renal failure patients without IRE or FID. In quadrant 2 there may be reduced iron supply but erythropoiesis is not yet iron deficient and haemoglobinisation of the red cells remains normal. Included in this quadrant are non-anaemic patients with latent iron deficiency, patients with iron deficiency shortly after commencing oral iron therapy, patients with hyperproliferative erythropoiesis due to acute haemorrhage, haemolysis and in the third trimester of pregnancy with increased sTfR but no IRE. Points in quadrant 3 suggest reduced iron supply as being the cause of IRE attributable to depleted iron stores as typically occurs in classical IDA. Data points in quadrant 4 occur in iron replete patients with the combined state of IRE/ACD who have anaemia accompanying infection or chronic inflammation and in the APR that accompanies cancer-related anaemia. Patients with β-thalassaemia trait have also points in quadrant 4. The diagnostic plot shows a good selectivity to assess the iron status of disease specific anaemias like classical ID, endstage renal failure, cancer related anaemia and anaemia of infection and inflammation (6).

Therapeutic implications of the diagnostic plot

Therapeutic implications of the diagnostic plot are to differentiate patients who should be administered with oral iron, with r-HuEPO or a combination of r-HuEPO and iron (*Figure 5*). Anaemic patients with data points in quadrants 2 and 3 are recommended for oral iron supplementation to the clinician. Usually in adequate oral iron supplementation patients respond with movement of data point from quadrant 3 into quadrant 2 within 10 days and into quadrant 1 after 4 weeks.

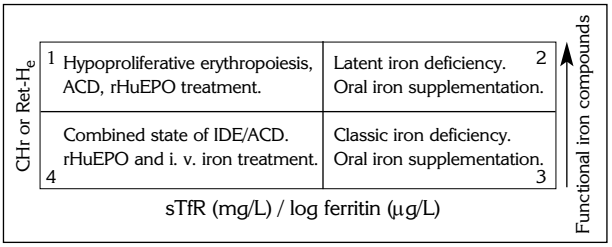

Figure 5. Therapeutic implications for treatment of different phases of iron deficiency

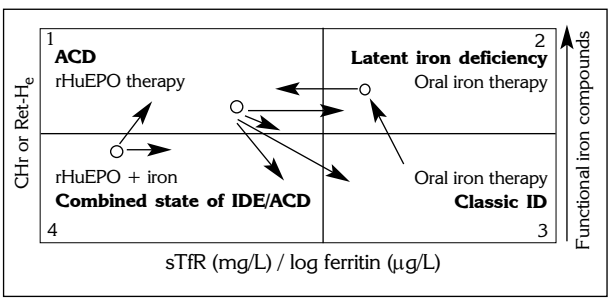

Figure 6. Response to treatment of anaemia

Anaemic patients with data points in quadrants 1 and 4 are recommended for r-HuEPO therapy. Patients with data points in quadrant 4 need intravenous iron supplementation with the first r-HuEPO dosage, patients with data points in quadrant 1 should be treated primarily only with r-HuEPO. The response of erythropoiesis to r-HuEPO is indicated by movement of the data points within the plot (*Figure 6*). An increase in CHr within a quadrant indicates sufficient iron supply to erythropoiesis, the decrease is an indication of FID. A movement of the data points into quadrants 2 and 3 is a sign of storage iron deficiency and a response to r-HuEPO.

In conclusion, biochemical markers of ID are only of value in diagnosing iron-restricted erythropoiesis in patients without APR. The combination reticulocyte indices CHr or RET-Y (RET-H_e) with the sTfR-F index in a diagnostic plot offers an attractive tool for identification of different phases of ID (14) and therapeutic monitoring of IRE.

BIOHEMIJSKI MARKERI I HEMATOLOŠKA INDIKACIJA ZA DIJAGNOZU I ERITROPOEZU OGRANIČENU GVOŽĐEM I PRAĆENJE r-HuEPO TERAPIJE

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Kratak sadržaj: Klasični nedostatak gvožđa (iron deficiency, ID) nije izazov za laboratoriju i lekare. Dijagnoza se postavlja na osnovu mikrocitne hipohromne anemije i niskog serumskog feritina. Anemija koja je praćena infekcijom, inflamacijom i kancerom, koja se najčešće označava kao anemija hroničnih poremećaja (anemia of chronic disorders, ACD) ima prividno normalne ili povećane rezerve gvožđa. Međutim, 20% ovih pacijenta ima eritropoezu ograničenu gvođžem (funkcionalna ID) i nesrazmeru između potreba za gvožđem od eritroidne srži i stvaranog snebdevanja gvožđem. Funkcionalna ID vodi kao smanjenju hemoglobinizacije eritrocita što izaziva hipohromnu mikrocitnu anemiju. Rana dijagnoza funkcionalne ID zasniva se na merenju sadržaja hemoglobina u ritikulocitima. Ovaj indeks može da se odredi merenjem CHr na Ter-Y analizatoru Bayer Advia i pomoću analizatora serije Sysmex XE. CHr < 28 pg ili Ret-Y < 1630 su osetljivi indikatori fukcionalne ID. Za procenu statusa gvožđe i otkrivanje uznapredovalog deficita gvožđe u pacijenta sa ID, ACD i kombinovanog stanja ID/ACD razvijeni su dijagnostički dijagrami. Dijagram ukazuje na korelaciju između odnosa sTfR/log feritin (feritin indeks), markera snabdevanja eritropoeze gvožđem, i CHr ili Ret-Y. Dijagnostički dijagram ukazuje na dobru selektivnost za procenu statusa gvožđa u oboljenju specifične anemije kao što je klasična ID, krajnjeg stadijuma oštećenja funkcije bubrega, anemije povezane sa karcinomom i anemije usled infekcija ili inflamacija. Terapeutske implikacije dijagnostičkog dijagrama su diferenciranje pacijenta kojima treba da se ordinira gvožđe oralno, r-HuEPO ili kombinacija r-HuEPO terapije, a što se prati korišćenjem Chr, REt-Y, sTfR ili dijagnostičkog dijagrama. Povećanje Chr > 1 pg, sTfR > 20% ili ushodno povećanje pacijentove dijagnostičke tačke na dijagramu ukazuje na odgovor na gvožđe ili r-HuEPO.

Ključne reči: anemija hroničnog oboljenja, retikulocitni sadržaj hemoglobina, funkcionalna deficijencija gvožđa, dijagnostički dijagram

References

1. Kendall RG. Erythropoietin. Clin Lab Haem 2001; 23: 71–80.
2. Means RT Jr, Krantz SB. Progress in understanding the pathogenesis of anemia of chronic disease. Blood 1992; 80: 1639–47.
3. Fishbane S, Shapiro W, Dutka P, Valenzuela OF, Faulbert J. A randomized trial of iron deficiency testing strategies in hemodialysis patients. Kidney Int 2001; 60: 2406–11.
4. Weiss G. Iron an anemia of chronic disease. Kidney Int 1999; 55 Suppl 69: S12–S17.
5. Israels LG, Israels ED, eds. Mechanisms in haematology. Winnipeg; The University of Manitoba, 1997: Section 6–1.
6. Thomas L, Thomas C. Biochemical markers and hematologic indices in the diagnosis of functional iron deficiency. Clin Chem 2002; 48:1066–76.
7. Cavill I. Functional iron deficiency. Blood 1993; 82: 137.
8. Brugnara C, Colella GM, Cremins J, Langley RC Jr, Schneider T, et al. Effects of subcutaneous recombinant human erythropoietin in normal subjects: Development of decreased reticulocyte hemoglobin content and iron-deficient erythropoiesis. J Lab Clin Med 1994; 123: 660–7.
9. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clin Chim Acta 2003; 329: 9–22.
10. Punnonen K, Irjala K, Rajarnäki A: Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood 1997; 89: 1052–7.
11. Brugnara C. Reticulocyte cellular indices: a new approach in the diagnosis of anemias and monitoring of erythropoietic function. Crit Rev Clin Lab Sci 2000; 97: 93–130.
12. Briggs C, Rogers R, Thompson B, Machin SJ. New red cell parameters on the Sysmex XE-2100 as potential markers of functional iron deficiency. Infus Ther Transfus Med 2001; 28: 256–62.
13. Franck S, Linssen J, Messinger M, Thomas L. Clinical utility of the RET-Y in iron-restricted erythropoiesis. Clin Chem 2004; 50: July.
14. Brugnara C. Iron deficiency and erythropoiesis: new diagnostic approaches. Clin Chem 2003; 49: 1573–8.

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