11.

Clinical Utility of the RET-Y in Functional Iron Defiency

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Introduction

Iron balance is fundamentally regulated by the rate of erythropoiesis and the size of the iron stores. In iron deficiency anaemia, the iron supply depends on the quantity of storage iron in the body whereas in functional iron deficiency, the supply depends on the rate of mobilisation of iron. Functional iron deficiency is therefore defined as an imbalance between the iron needs of erythropoiesis and the iron supply that is not maintained at a sufficient rate to permit normal haemoglobinisation of reticulocytes and mature erythrocytes in spite of adequate body stores of iron. It is well known that patients with the anaemia of chronic disease (ACD) have replete iron stores and have ferritin levels ranging from 200 – 500 µg/L, but nevertheless about 20% of these patients have functional iron deficiency.

Conventional laboratory tests for iron status include serum iron, transferrin/total iron-binding capacity, transferrin saturation and ferritin. Although widely used, these parameters are influenced by the acute phase response which complicates clinical interpretation of the test results. Ferritin is an acute phase reactant. The soluble transferrin receptor (sTfR) assay is yet another promising tool for the diagnosis of iron depletion. Calculation of the ratio sTfR/log ferritin (sTfR/F or Ferritin Index) is a way of combining sTfR and ferritin results [**1**]. More recently Thomas and Thomas [**2**] have assessed the extent to which biochemical markers can distinguish iron deficiency from the anaemia of chronic disease and from the combined state of functional iron deficiency/anaemia of chronic disease using red cell haemoglobinisation as a 'gold standard'. The haemoglobin content of reticulocytes and mature red blood cells provides direct evaluation of the bone marrow activity reflecting the balance between iron and erythropoiesis. Modern haematology analysers capable of identifying small sub-populations of erythrocytes within the total RBC population offer appropriate tools.

Haematological indices which have gained merit in the assessment of functional iron status include the reticulocyte haemoglobin content (CHr) [**3**] and the proportion of hypochromic red cells (%HYPO) [**4**]. Until recently both haematology parameters have been restricted to the analysers of a single manufacturer. Now a second manufacturer would appear to have produced an equivalent parameter, the so-called RET-Y [**5**].

The objectives of this presentation are twofold: (1) to describe a diagnostic plot combining haematological indices with the ferritin index as a tool for the diagnosis and therapeutic monitoring of functional iron deficiency, and (2) to establish the clinical equivalence of the RET-Y with the CHr.

The Diagnostic Plot

The rationale behind the creation of the diagnostic plot is described in detail by Thomas and Thomas [**2**]. Initial studies involved the evaluation of more than 600 patients comparing the ferritin index (ratio of sTfR [mg/L] / log ferritin [µg/L]) with the haemoglobin content of the reticulocytes (CHr). The diagnostic plot is illustrated in **figure 1**. The sTfR level was measured using the Dade assay. From our studies we found that patients with a ferritin index higher than 1.5 have iron-depleted stores while patients with an index less than 1.5 have stores which are iron replete. Patients with a CHr level greater than 28 pg or HYPO less than 5% (normal haemoglobinisation of red cells and reticulocytes) do not have functional iron deficiency whereas those with CHr less than 28 pg or HYPO greater than 5% (reduced haemoglobinisation of red cells and reticulocytes) have functional iron deficiency. It is also important to know whether or not an inflammatory disorder co-exists. The C-reactive protein (CRP) assay was used as a marker of inflammation, all patients with a CRP level greater than 5mg/L being considered to have an inflammatory disorder. For patients without inflammation, the ferritin index separating iron depleted from an iron replete state is 1.5 as previously stated. However, in patients with elevated CRP levels, the decision point is 0.8 since ferritin is an acute phase protein and is elevated in inflammatory disorders independently of the body iron stores. As a result the *log*-ferritin is increased and the decision point is moved to a ratio of 0.8.

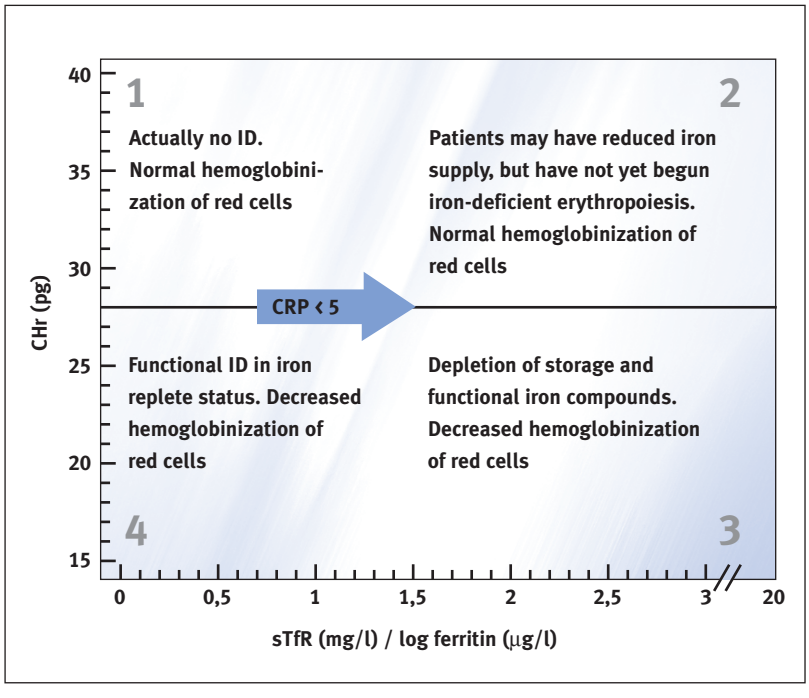

Figure 1
The diagnostic plot described by Thomas and Thomas (2).
ID = iron deficiency
CHr = reticulocyte haemoglobin,
sTfR/log-ferritin = sTfR-F index (1).

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 haemoglobinisation. In this quadrant are located patients with cancer-related anaemia (CRA), anaemia of chronic disease (ACD), and end stage renal failure patients without functional iron deficiency. 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 functional iron deficiency. Points in quadrant 3 suggest reduced iron supply being the cause of functional iron deficiency attributable to depleted iron stores as typically occurs in iron deficiency anaemia. Data points in quadrant 4 occur in iron replete patients with functional iron deficiency anaemia who have anaemia accompanying infection or chronic inflammation and in the acute phase response that accompanies cancer-related anaemia. Patients with β-thalassaemia trait and those with combined iron deficiency/anaemia of chronic disease have points in quadrant 4.

We use the diagnostic plot in our hospital to give recommendations to the clinicians. When a patient point lies in either quadrant 2 or quadrant 3 it is recommended that the patient be treated with oral iron supplements. If the patient point is in quadrant 1 and the patient is anaemic with a haemoglobin level less than 8–10 g/dL we recommend erythropoietin therapy provided the patient is not receiving iron. During the erythropoietin therapy we monitor the CHr level and if it falls below 28 pg, iron therapy is recommended. If the CHr does no fall below 28 pg, iron is not given. If the points are in quadrant 4, the recommendation is for both erythropoietin and iron. If the patient responds after 8–10 days, then the point may move from quadrant 1 or 4 to quadrant 2. This diagnostic plot has been used successfully in thousands of patients and clinicians have been very satisfied with the recommendations arising from its use.

Comparison of RET-Y with CHr in the diagnostic plot

Initially only CHr was available and this limited the usefulness of the diagnostic plot, therefore when the RET-Y became available on the analyser from another manufacturer we were keen to evaluate this. Our task was to demonstrate whether or not the RET-Y was also reliable in the selected patient groups. Briggs *et al* [**5**] described a comparison of RET-Y with CHr two years ago and concluded that they measured the same phenomenon.

In the reticulocyte channel of the SYSMEX XE-2100, the sample, stained by a polymethine dye specific for RNA/DNA, is analysed by flow cytometry using a semiconductor laser. A two-dimensional distribution of forward scattered light (y-axis) and fluorescence (x-axis) is presented as a scattergram (**figure 2**) with mature red blood cells represented in the blue cloud and reticulocytes in the red cloud. RET-Y is the mean value of the forward scattered light histogram of the reticulocyte population.

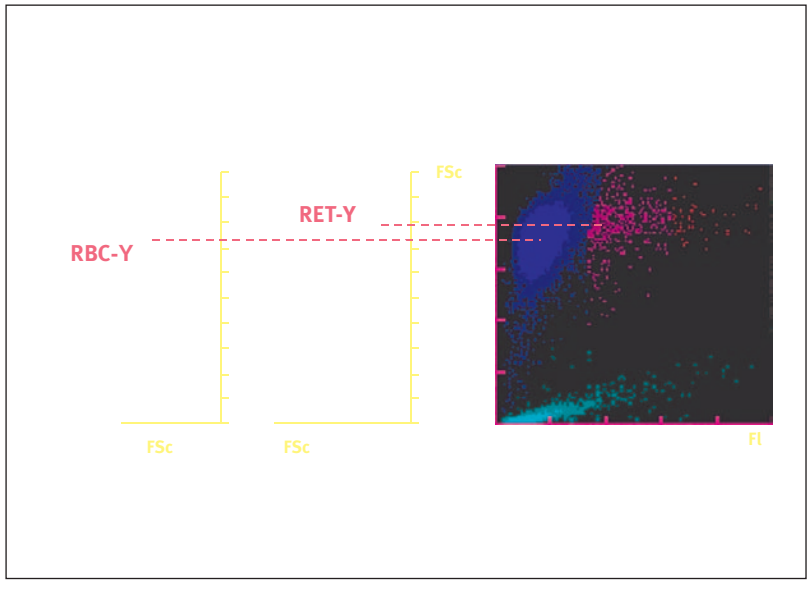

Figure 2
Reticulocyte channel. Determination of the RBC-Y and the RET-Y on the SYSMEX XE-2100. RBC-Y is the mean value of the forward-scattered light histogram within the mature red cell population and RET-Y is the mean value within the reticulocyte population.

First we compared RET-Y with CHr in 463 patients and demonstrated an excellent curvilinear relationship (**figure 3**) between the two parameters with $r^2 = 0.924$.

The next study involved splitting those 463 patients divided into two groups: (1) those without acute phase response (138 patients) and (2) those with acute phase response (325 patients) and comparing CHr with sTfR-F index.

Applying those patients without acute phase response (**figure 4**) to the diagnostic plot, most fall into quadrant 1 meaning that there is no disturbance of iron metabolism. Patients with data points in quadrant 3 have classical iron deficiency. Only a small number of patients had data points in quadrant 4 indicating that the combined state of functional iron deficiency with the ACD is seldom seen in patients with normal CRP.

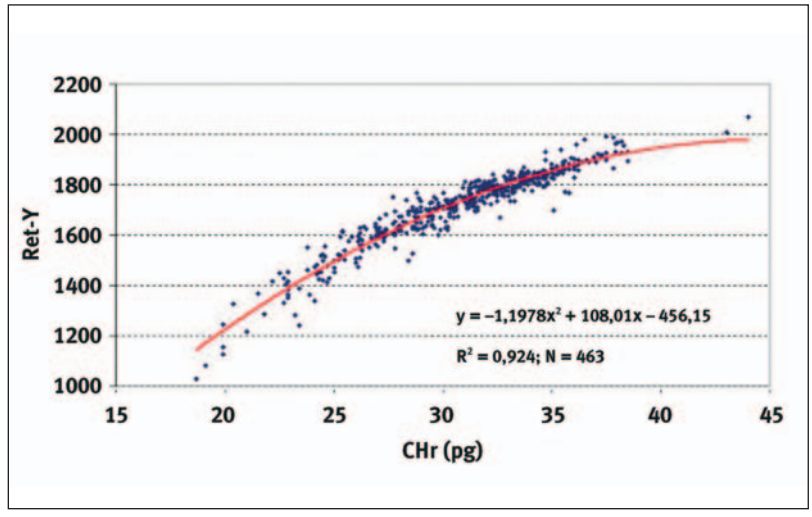

Figure 3
Relationship between the RET-Y and the CHr.

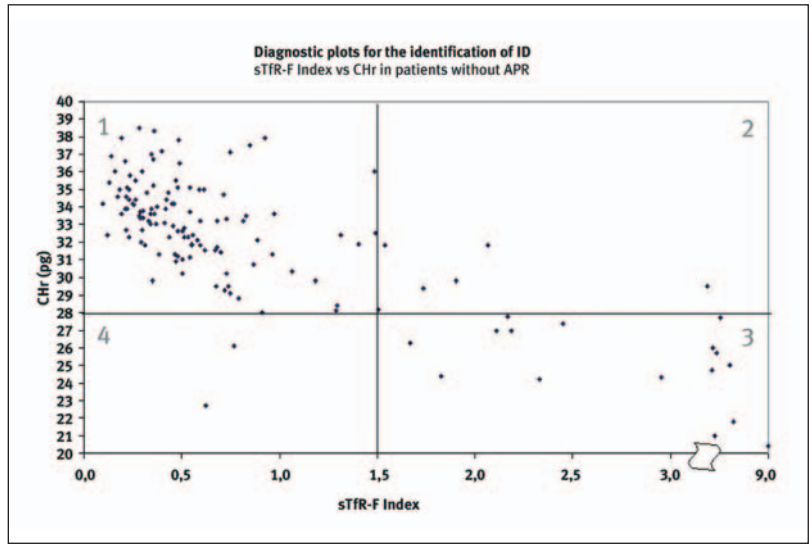

Figure 4
Application of patients without acute phase response to the Diagnostic Plot.

Turning now to the patients with acute phase response (**figure 5**), most had CRP values between 5 and 300 mg/L with a median value of 35 mg/L. Perhaps 15–20% of patients in this group fall in quadrant 4, i.e., they have functional iron deficiency but are in iron-replete status. All patients in quadrant 3 and 4 have at least two of four criteria indicating functional iron deficiency: % HYPO › 5, CHr ‹ 28 pg, a CH inversion (CHr ‹ CH of red cells), elevation of sTfR.

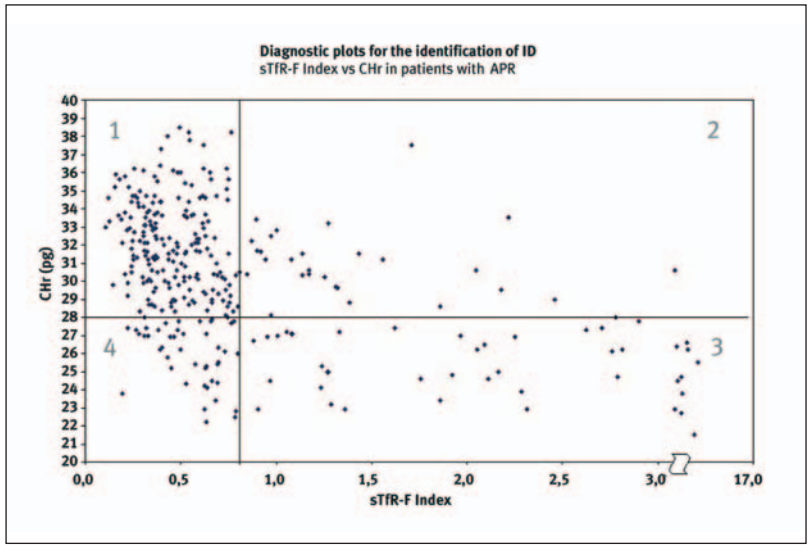

Figure 5
Application of patients with acute phase response to the Diagnostic Plot.

If CHr is replaced by RET-Y on the y-axis of the diagnostic plot and these two patient groups re-analysed, the results are virtually identical (**figure 6** and **7**).

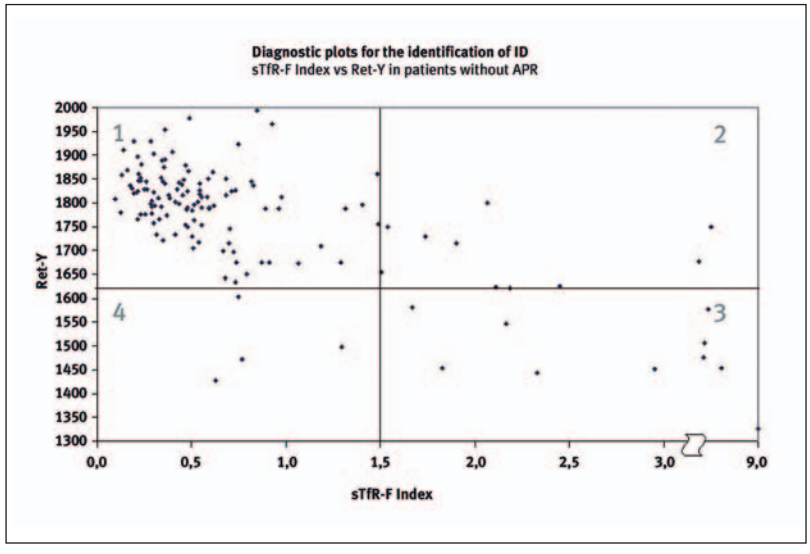

Figure 6
Application of patients without acute phase response to the Diagnostic Plot. Note the CHr on the y-axis has been replaced by RET-Y.

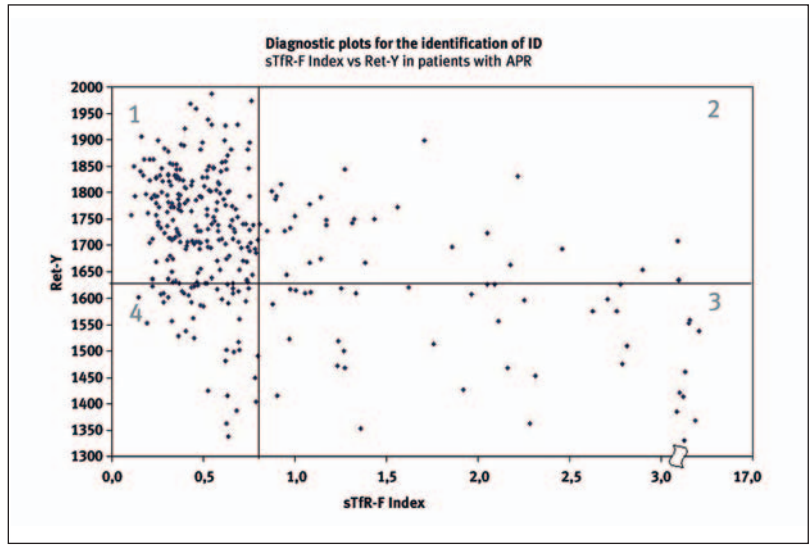

Figure 7
Application of patients with acute phase response to the Diagnostic Plot. Note the CHr on the y-axis has been replaced by RET-Y.

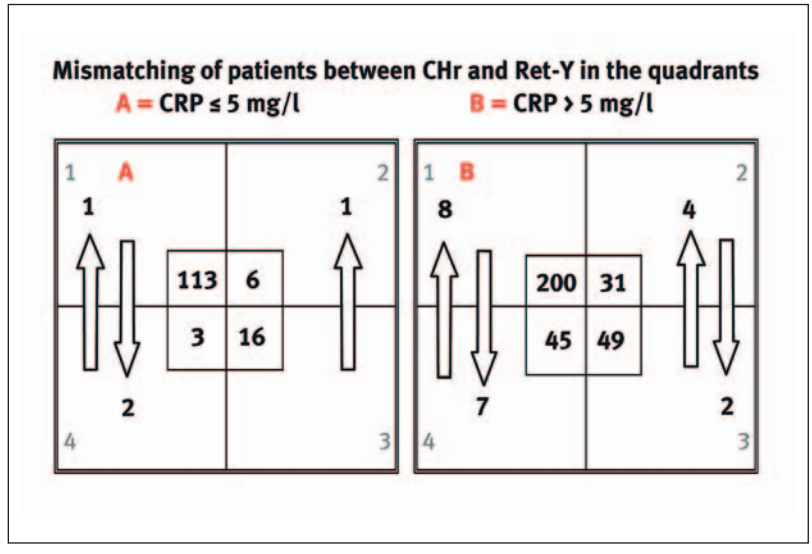

Figure 8
Patient mis-match between CHr and RET-Y in each of the quadrants. A = CRP ≤ 5mg/L; B = CRP ≥ 5mg/L.

Patient mismatch analysis between CHr and RET-Y in the four quadrants is shown in **figure 8**. Of the 138 patients without acute phase response, RET-Y mis-matched only one patient from quadrant 4 to quadrant 1, two patients from quadrant 1 to quadrant 4, and 1 patient from quadrant 3 to quadrant 2. Of the 325 patients with acute phase response, RET-Y mismatched only eight patients from quadrant 4 to quadrant 1, seven patients from quadrant 1 to quadrant 4, four patients from quadrant 3 to quadrant 2, and two patients from quadrant 2 to quadrant 3. This means that RET-Y shows more than 95 % agreement with CHr in placing patients in the correct quadrant. Patient-specific anaemia mismatches are presented in **table 1**

	Pregnancy	ERF	CRA	Infection	Hetero
Number	2/34	5/142	11/161	2/48	4/78
%	5.9	3.5	6.8	4.2	5.1

Table 1
Mismatches of specific anaemias between CHr and RET-Y.
ERF = end stage renal failure;
CRA = cancer-related anaemia;
Hetero = heterogeneous diseases.

The final study involved assessing how the haematological indices correlated with the biochemistry indices. First, in patients without an acute phase response (**figure 9**) there is a reasonably reliable correlation between CHr and sTfR with $r^2 = 0.6679$. This deteriorates to $r^2 = 0.353$ for patients with acute phase response (**figure 10**). Corresponding correlations for RET-Y and sTfR are $r^2 = 0.5701$ (**figure 11**) and $r^2 = 0.4151$ (**figure 12**) respectively without and with acute phase response.

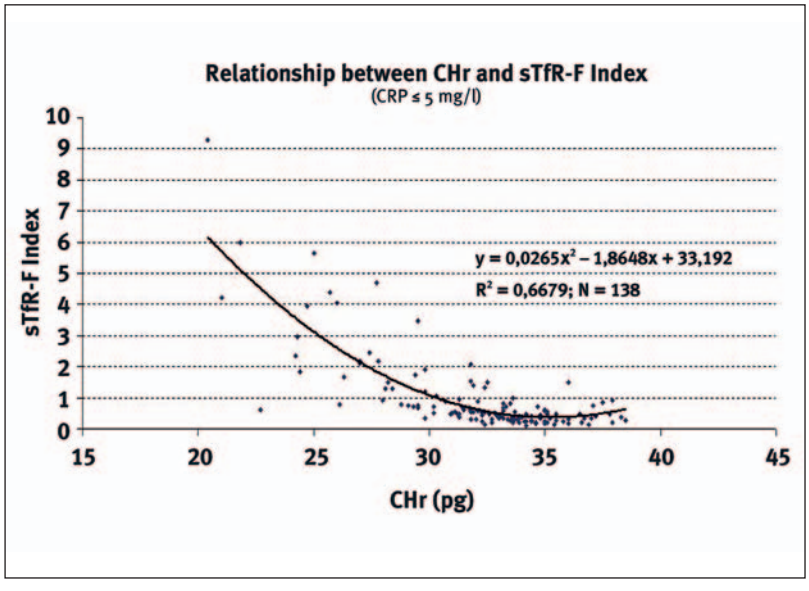

Figure 9
Relationship between CHr and sTfR-F Index. CRP ≤ 5mg/L.

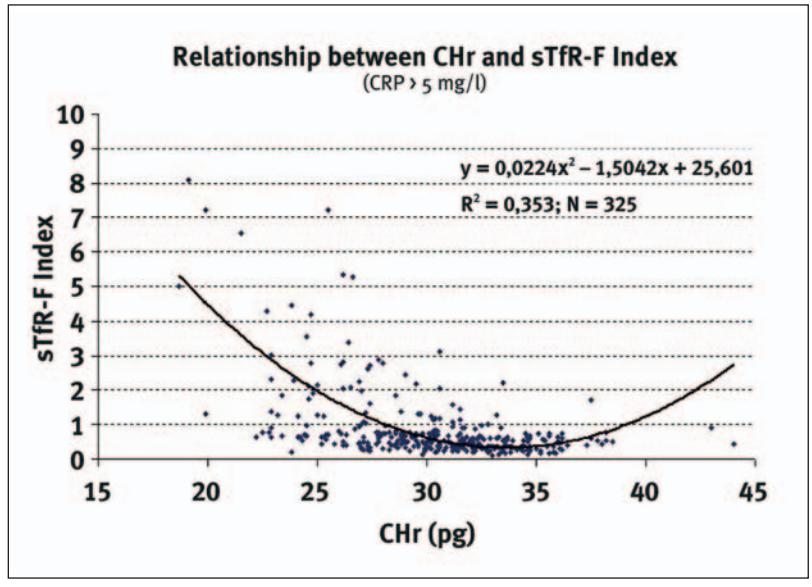

Figure 10
Relationship between CHr and sTfR-F Index. CRP ≥5mg/L.

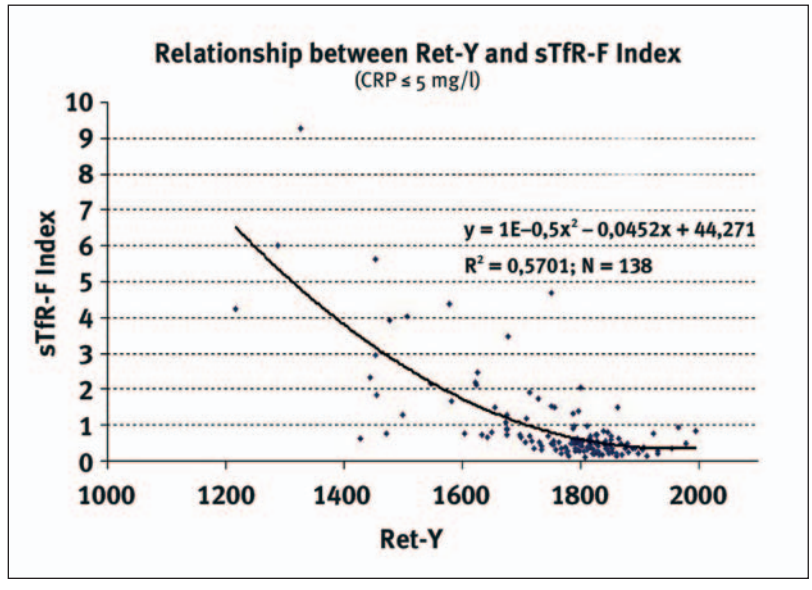

Figure 11
Relationship between RET-Y and sTfR-F Index. CRP ≤ 5mg/L.

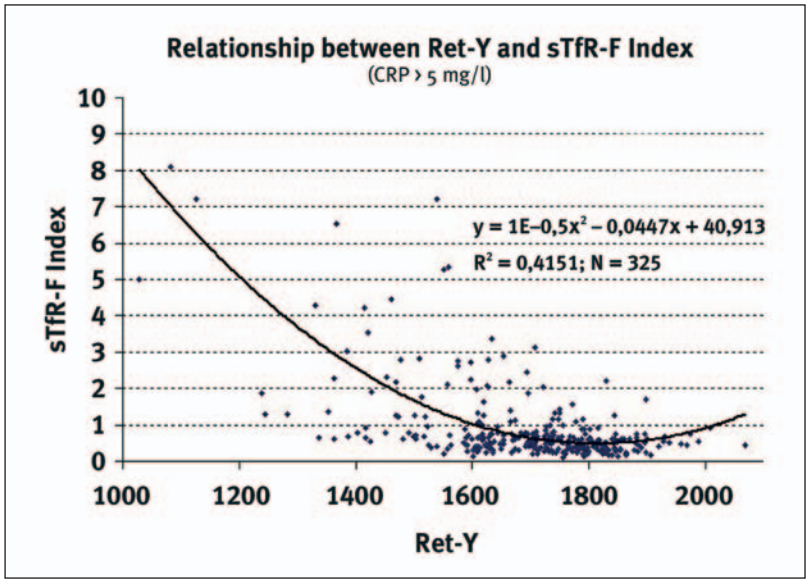

Figure 12
Relationship between RET-Y and sTfR-F Index. CRP ≥5mg/L.

Conclusions

Classic iron markers are of limited value in diagnosing functional iron deficiency especially in diseases associated with an acute phase response. Correlations with the haematological indices CHr and RET-Y are weak in the presence of acute phase response. The present study shows that the parameters CHr and RET-Y are measuring the same phenomenon and the combination of either in a diagnostic plot offers an attractive tool for the diagnosis and monitoring of functional iron deficiency (**figure 13**).

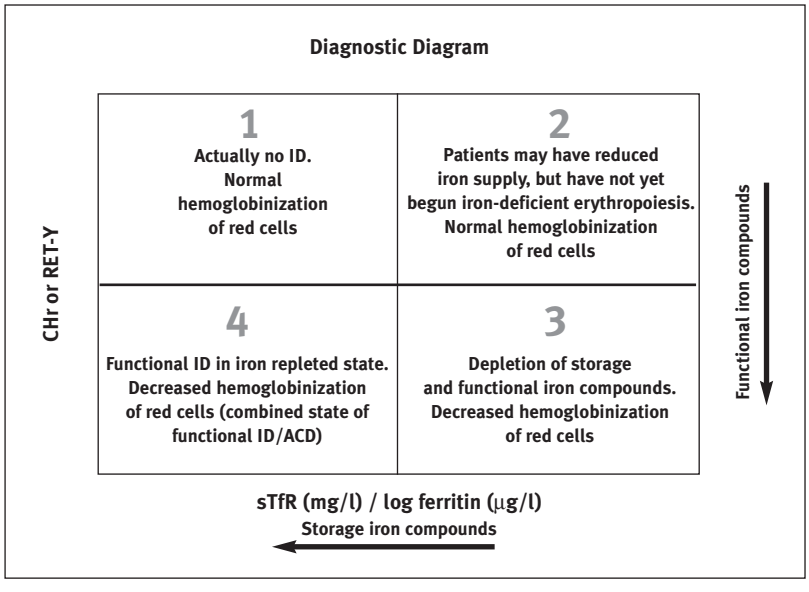

Figure 13
Diagnostic Plot showing CHr and RET-Y as valid alternatives on the y-axis.

References

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