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The usefulness of the serum transferrin receptor in detecting iron deficiency in the anemia of chronic disorders

JORDI JUNCÀ, FRANCESC FERNÁNDEZ-AVILÉS, ALBERT ORIOL,* JOSEP TOMAS NAVARRO, FUENSANTA MILLÁ, JUAN M. SANCHO, EVARIST FELIU

*Hematology Departments, Hospital Universitari Germans Trias i Pujol and *Hospital Municipal, Badalona, Spain*

Abstract

Background and Objective. **Recent studies have shown that the serum transferrin receptor (sTfR) is a sensitive, quantitative measurement of tissue iron deficiency. The objective of the study was to evaluate the diagnostic efficiency of some laboratory tests, including sTfR measurements, in the diagnosis of iron depletion in patients with anemia of chronic disorders.**

Design and Methods. **The patient population consisted of 37 anemic patients: 10 hypoferritinemic patients (serum ferritin < 25 µg/L), and 27 anemic in-patients with hyperferritinemia (serum ferritin >200 µg/L) and clinical/analytical criteria of anemia of chronic disorders, who were submitted to a bone marrow aspirate with iron stain. The sensitivity and specificity of serum TfR was evaluated according to the results of bone marrow iron status. Statistical analysis employed Student's t-test, one way analysis of variance and a logistic regression model using the Wald test.**

Results. **Serum TfR was high in all the patients with hypoferritinemic anemia. In 12 patients with low bone marrow iron, the mean sTfR was 5.63 mg/L. In 6 of these 12 patients the sTfR was normal. On the other hand, sTfR was high in 4/15 patients with normal or increased iron stores. On multivariate analysis the most sensitive predictor of true iron deficiency was MCH (mean corpuscular hemoglobin). No other variables remained independently significant, including sTfR, after the inclusion of MCH in this model.**

Interpretation and Conclusions. **In our opinion, the iron status of patients with anemia of chronic diseases can not be accurately assessed by sTfR, as its sensitivity and specificity are low. In these patients, the *gold standard* for iron stores evaluation continues to be bone marrow aspirate and Perls stain.**

Key words: serum transferrin receptor, bone marrow stainable iron, anemia of chronic disorders, iron deficiency

Correspondence: Jordi Juncà, MD, Servei d'Hematologia, Hospital Germans Trias i Pujol, Ctra. Canyet s/n, 08916 Badalona (Barcelona), Spain.
Phone: international +34-93-4651200 • Fax: international + 34-93-3954206 • E-mail: jjunca@ns.hugtip.scs.es

Iron metabolism disorders account for most of the anemias seen in hospitalized patients. In this setting, the two main types of anemia are iron deficiency anemia and the so-called anemia of chronic disorders (ACD).[1] Although *pure* forms of both may be readily distinguished, in an important number of cases both diseases coexist, making it difficult to ascertain whether the main cause of the anemia in a given patient is iron deficiency, masked by an inflammatory, infectious or degenerative state, or if the anemia is, in itself, due to this state.

Classical analytical parameters are of little help in making this distinction as the acute phase reaction can alter the behavior of most of the analytes: serum iron and transferrin decrease and serum ferritin increases,[2] thus the predictive value of these parameters is low. Attempts have been made to increase the diagnostic value of serum ferritin by establishing a nomogram which relates its concentration with some acute phase reactants, namely ESR, but the diagnostic accuracy of this maneuver has been questioned.[3-5] In this ambigous situation bone marrow examination, along with Perls stain, has been considered the *gold standard* to decide whether iron deficiency plays a role in the origin of the anemia, although it has been claimed that the quantification of serum transferrin receptor (sTfR)[6] could be used instead of bone marrow aspiration, thereby avoiding its inconveniences. Thus, as some authors have stated, serum levels of sTfR could allow the distinction between iron deficiency and ACD to be made,[7-11] although this has been questioned by others.[12-13]

The aim of our study was to assess the clinical usefulness of the determination of sTfR in the diagnosis of *masked* iron deficiency in the context of an inflammatory or infectious situation.

Patients and Methods

Patients

Three different groups of patients were studied: 10 hypoferritinemic patients (mean age 65 years, 8 females, serum ferritin < 25 µg/L) with anemia secondary to chronic blood losses (Hb < 120 g/L) who acted as controls of the sTfR technique; and 27 anemic in-patients (mean age 65.7 years, 14 women),

with an infectious or inflammatory disease (pneumonia 7 cases, pericarditis 2 cases, SLE 3 cases, Still's disease 2 cases, polymyalgia rheumatica 3 cases, rheumatoid arthritis 3 cases, septicemia 2 cases, fever of unknown origin 5 cases). All of these patients had hyperferritinemia (serum ferritin >200 µg/L). The anemia of these 27 patients was microcytic (MCV < 82 fL) in 11 cases and normocytic (MCV>82 fL) in the remaining 16 cases.

In a second diagnostic step all of these 27 patients with hyperferritinemia were submitted to bone marrow aspiration for evaluation of iron stores, and were then allocated into one of these two groups: LI (*low* bone marrow *iron*) Group (12 patients) or HI (*normal* or *increased* bone marrow *iron*) Group (15 patients), regardless of other analytical parameters (MCV, MCH, free erythrocyte protoporphyrin, ESR, C-reactive protein, fibrinogen).

Methods

Hematimetric data were obtained with a Coulter MAXM or a Technicon H2 counter (Bayer). ESR, C reactive protein (CRP) and fibrinogen were measured by conventional methods. Ferritin was measured with an enzyme linked fluorescent assay (VIDAS Ferritin, BioMérieux, France), free erythrocyte protoporphyrin (FEP) was determined with an hematofluorometer (AVIV model 206, USA), and sTfR with an immunoenzymometric assay (IDeA sTfR IEMA, Orion Diagnostica, Finland). Bone marrow smears were stained with May Grünwald-Giemsa for global analysis and with Prussian Blue stain (HematoGnost, Diagnostica Merck, Darmstatd, Germany) for iron stores evaluation and sideroblasts counting. Each slide was examined by two different observers with the final consensus of marrow iron grade being recorded as 0 (absent), + (reduced), ++ (normal) or +++ (increased). These records were simplified into only two categories (absent or low vs normal or high).

The Student's t-test was used to compare the variables between the two groups. One-way analysis of variance was used when simultaneous comparison of three groups was required. Relevant variables in univariant analysis were included as predictors for iron deficiency in a logistic regression model and excluded in a backward stepwise fashion if lacking independent significance in a Wald test.

Table 1. Comparison of iron status parameters between patient groups with *low* bone marrow *iron* (LI) and *normal* or *increased* bone marrow iron (HI).

Variable	*LI Group (N=12) Mean (SD)*	*HI Group (N=15) Mean (SD)*	*p-value* for 2 groups*
Age (years)	62.33 (19.7)	68.47 (15.9)	NS
Hemoglobin (120-160 g/L)	9.37 (1.5)	8.17 (1.5)	0.0436
MCV (82.0-95.0 fL)	78.38 (7.5)	87.65 (7.1)	0.0031
MCH (28.0-32.0 pg)	25.07 (3.3)	28.28 (3.0)	0.0137
ESR (<15 mm/hour)	70.30 (41.8)	90.57 (31.4)	NS
Fibrinogen (150-450 mg/dL)	727.20 (206.6)	758.33 (288.9)	NS
FEP (0.0-3.0 µg/gHb)	7.56 (9.1)	4.21 (2.5)	NS
STfR (3.1-4.5 mg/L)	5.63 (3.3)	3.39 (1.9)	0.036
Ferritin° (25.0-200.0 µg/L)	506.75 (454.4)	1570.80 (1780.2)	NS

**p-value for Student's T-test. MCV: mean corpuscular volume. MCH: mean corpuscular hemoglobin. ESR: erythrocyte sedimentation rate. FEP: free erythrocyte protoporphyrin. sTfR: serum transferrin receptor. °Serum ferritin for women, range 25-90 µg/L; for men, range 50-200 µg/L.*

Results

Relevant clinical and analytical data for groups *Low Iron* and *High Iron* are shown in Table 1. Significant differences in Hb, MCV, MCH, and serum concentration of sTfR and ferritin were observed among these two groups (both with high serum ferritin): low MCV (p=0.0031), low MCH (p=0.0137) and high sTfR (p=0.036) values remained significantly associated with iron deficiency. Hemoglobin appeared significantly lower in the HI group than in the LI group (p = 0.0436) although both groups had higher hemoglobin levels than the group of 10 patients with hypoferritinemia due to chronic blood loses. The MCV was lower than 82 fL and the MCH lower than 28 pg in 7/12 and 10/12 patients, respectively, of the LI group, and in 4/15 and 6/15 patients of the HI group.

The sTfR presented significant linear inverse correlations with both MCV ($r = -0.61$; $p < 0.001$) and MCH ($r = -0.74$; $p < 0.001$) as well as with ferritin ($r = -0.41$; $p = 0.011$). To establish the relative predictive value of these parameters and specifically determine the additional value of sTfR in the diagnosis of iron deficiency, multivariate logistic regression analysis was performed. A low MCH (best cutpoint MCH < 28 pg) was the best predictive parameter of iron deficiency. The odds ratio for the risk of iron deficiency with a low MCH was 16.5 (95% confidence interval 1.69 to 159.75). Using this parameter 20 of the 27 patients studied (74%) were correctly classified (60% of non-iron deficient and 91.67% iron deficient). No other variables remained independently significant after inclusion of MCH in the model. Specifically, sTfR was the first variable to be excluded from the model as non-significant (Wald test) and it did not improve its predictive value at any cut-off point. Furthermore, sTfR was found to be high, reflecting iron deficiency, in only 6 out of 12 patients

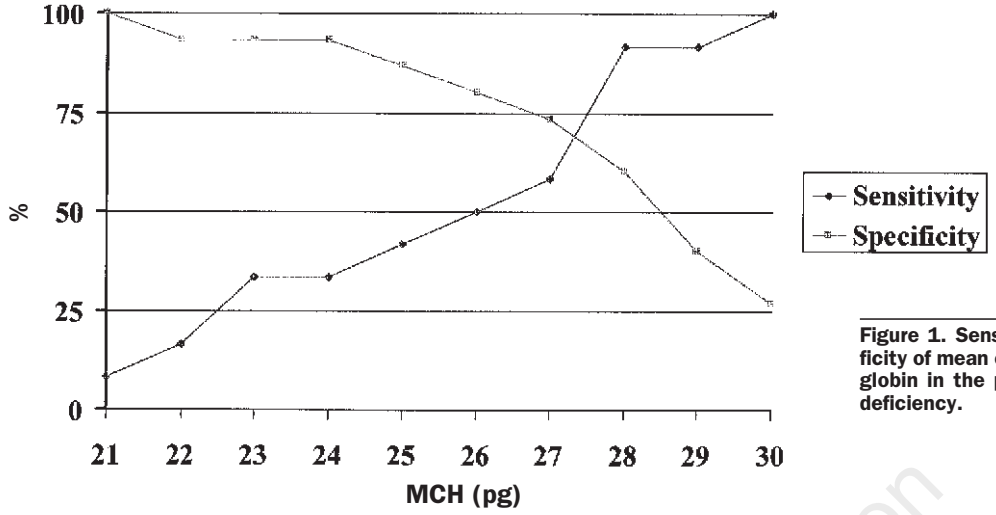

Figure 1. Sensitivity and specificity of mean corpuscuar hemoglobin in the prediction of iron deficiency.

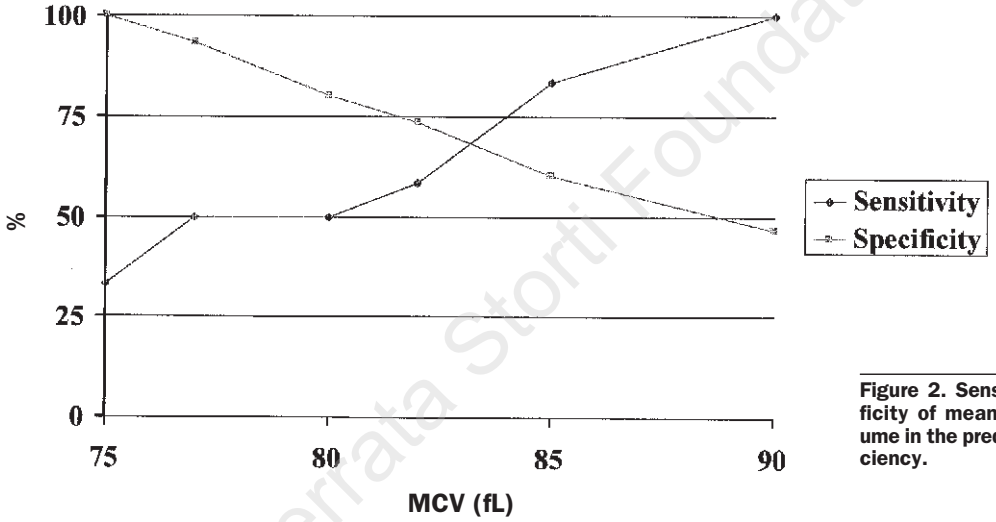

Figure 2. Sensitivity and specificity of mean corpuscular volume in the prediction of iron deficiency.

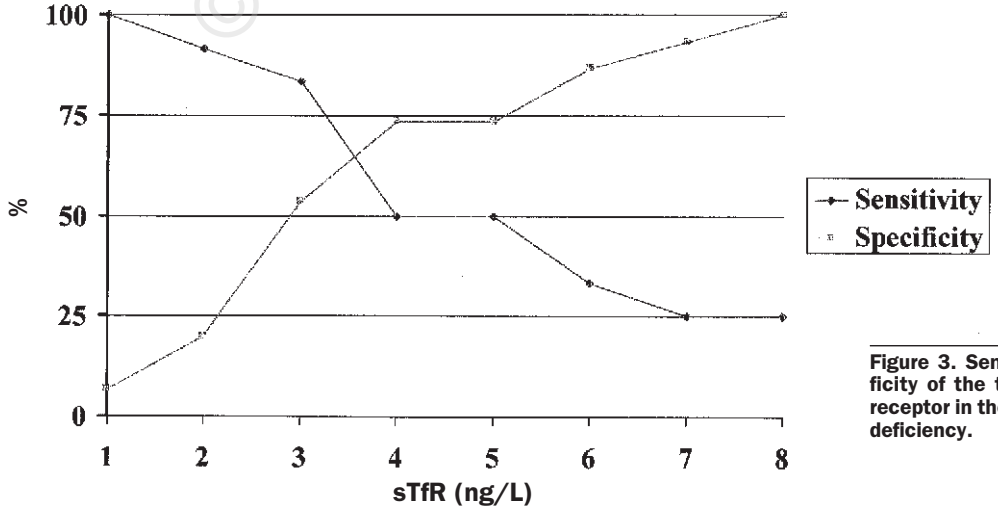

Figure 3. Sensitivity and specificity of the transferrin soluble receptor in the prediction of iron deficiency.

without stainable bone marrow iron. On the other hand, sTfR was also elevated in 4 out of 15 patients with normal or increased bone marrow stores.

Sensitivity and specificity graphs for sTfR, MCH and MCV were analyzed to detect the best cut-off points to discriminate iron deficiency in groups LI and HI (Figures 1, 2 and 3). No linear combinations of two or three of the original variables or their logarithmic transformations added relevant diagnostic power to MCH alone.

Discussion

Iron deficiency is by far the most frequent cause of anemia, but in hospitalized patients the anemia that accompanies infections, inflammation and neoplasia [anemia of chronic disorders (ACD)] is also very frequent. Although in some cases the distinction among *pure* iron deficiency anemia (IDA) and ACD can be clearly made, on many occasions it can be very difficult to ascertain whether the anemia of a given patient is only due to ACD or whether there is an underlying IDA. This is due, at least in part, to the acute phase reaction that accompanies chronic (or subacute – acute) disorders.

Different strategies have been devised to avoid the direct visualization of iron stores performing a bone marrow aspirate. Witte *et al.*[3,4,14] claimed that bone marrow iron stores may be properly predicted when serum ferritin levels are interpreted along with ESR. However, these findings have not been confirmed by others. Coenen *et al.*[5] could not correctly diagnose the type of anemia (IDA *vs* ACD) with high ferritin concentrations taking ESR, CRP or fibrinogen values into account.

Later, it seemed that the quantification of sTfR[6] could obviate the need for performing bone marrow aspirate as some studies indicated that its increase in plasma would reflect a decrease in stainable iron. Therefore, Ferguson *et al*.[7] stated that sTfR could be of value in distinguishing between IDA and ACD as their levels remain normal in ACD. They did not, however, address the problem of the coexistence of iron deficiency and ACD. Furthermore, a bone marrow aspiration was performed in only 17 out of the 56 patients studied. This issue was addressed by Punnonen *et al:*[8] sTfR was high in 19 patients with no stainable iron but was normal or low in 17 patients with normal iron stores. According to their results, measurement of sTfR seemed "*to provide a means of identifying iron deficiency even in patients with acute phase reactions associated with inflammatory conditions*". These results were similar to those obtained by Petterson *et al*.,[10] who found that in 23 patients with active rheumatic disease sTfR levels could adequately predict bone marrow iron stores. These findings were questioned by others, such as Zoli *et al*.,[12] who did not detect any difference in sTfR levels depending on the normality of iron stores in 72 patients with active rheumatoid arthritis. Baumann *et al*.[13] obtained similar results with the highest specificity in predicting iron deficiency in chronic inflammatory rheumatic disease anemia being attained by the percentage of hypochromic red cells in peripheral blood or zinc bound protoporphyrin. Neither ferritin interpreted along with other acute phase reaction indicators nor sTfR allowed good evaluation of iron stores. In a more recent article, North *et al.*[11] found sTfR to be high in 4 out of 7 patients who fulfilled analytical criteria for the diagnosis of ACD, and no alternative explanation was found for this disagreement, which was also observed in our series of patients. The last work on this topic[9] concludes that sTfR determination may distinguish between isolated ACD and ACD plus IDA, and that the accuracy of sTfR may increase when the index sTfR/ferritin log is used instead of the isolated determination of sTfR.

As can be seen from this brief review of the literature, the controversy on the actual usefulness of sTfR determination is far from being solved as the results of different reports are disappointing. According to our results, iron deficiency in the context of ACD was detected by sTfR in only half of the patients without stainable iron. Furthermore, and even more surprising, sTfR was elevated in 4 out of 15 patients with normal or increased bone marrow iron, a finding previously described by North *et al.*[11] None of these 4 patients had erythroid hyperplasia, another possible explanation for the increase in sTfR concentration. On the other hand, when multivariate analysis was performed, the most sensitive predictive parameter for the presence of iron deficiency was the decrease in MCH, a finding similar to that described by Baumann *et al.*,[13] followed by a decrease in MCV. However, none of the analyzed variables retained any significance, including sTfR, after the inclusion of MCH in the model. According to that, a hemogram would act as a better predictor of true iron status than other more sophisticated techniques.

Given all these facts, we conclude that sTfR determination does not always reflect what it is really happening with bone marrow iron and that, up to now at least, the true status of iron stores is better assessed by means of bone marrow aspiration.

Contributions and Acknowledgments

JJ was the main investigator, designed the study and performed the literature revision; he wrote the article with FF-A, who contributed to its final writing with his suggestions. AO performed the statistical analysis. Bone marrow aspirates and biopsies were performed and interpreted by JTN, FM and JMS. EF contributed to the work with his final suggestions. The order tries to take into account the time work, and scientific contribution of all authors.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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