Discriminating Between Iron Deficiency Anemia and Anemia of Chronic Disease Using Traditional Indices of Iron Status vs Transferrin Receptor Concentration

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Key Words: Transferrin receptor; Iron deficiency anemia; Anemia of chronic disease; Iron studies; Ferritin; Receiver operating characteristic curve

Abstract

We compared the ability of soluble serum transferrin receptor (TfR) concentration, quantified using the R&D Systems (Minneapolis, MN) enzyme-linked immunosorbent TfR assay, with other, more traditional indicators of iron status (total iron binding capacity [TIBC], mean corpuscular volume [MCV], percent transferrin saturation [%TS], RBC distribution width [RDW], and serum iron concentration [SIC]) for discriminating between patients with iron deficiency anemia (IDA) or anemia of chronic disease (ACD). The TfR concentration was determined in 72 serum samples selected from men and nonpregnant women classified biochemically on the basis of ferritin concentration as having IDA (n = 41) or ACD (n = 31). By using receiver operating characteristic curve analysis, the diagnostic accuracy of the various indicators of iron status that we evaluated for discriminating between IDA and ACD decreased in the following order: TIBC > TfR > MCV > (%TS = RDW) > SIC. There was no significant difference between the diagnostic accuracy of TIBC and TfR. Thus, the routine measurement of TfR offers no advantage over TIBC for discriminating between people with biochemically defined IDA or ACD.

The cellular transferrin receptor (TfR) molecule is a transmembrane protein that binds transferrin, the principal iron transport protein found in the blood.1,2 TfR is found in highest concentration on the surface of cells requiring large amounts of iron, such as hemoglobin-synthesizing cells of the reticuloendothelial system (ie, bone marrow, liver, and spleen)3 and the placenta.4-7

In 1986, Kohgo et al8 were the first to demonstrate the presence of a soluble form of TfR in human serum that was identified subsequently as a truncated form of cellular TfR derived from proteolytic cleavage of its extracellular segment.9-11 Moreover, 80% of the total serum level of TfR originates from immature RBC progenitors, including reticulocytes, while 20% originates from nonerythroid tissues.1,4

Since most nonerythroid and nonhematologic malignant neoplasms are not associated with increased serum TfR levels,12 the principal clinical usefulness of TfR has been shown to be in the assessment of total erythropoiesis,12-14 in monitoring recovery of erythropoietic activity after bone marrow transplantation,1 in quantifying the response to therapy with human recombinant erythropoietin,15-17 in diagnosing “functional iron deficiency,”18-20 in distinguishing iron deficiency anemia (IDA) from anemia of chronic disease (ACD),21,22 and in the overall evaluation of iron status.23,24

The evaluation of iron status in patients with anemia typically includes assessment of values for the traditional quantitative laboratory tests: serum iron concentration (SIC), total iron binding capacity (TIBC, an indirect estimate of transferrin concentration), percent transferrin saturation [%TS = (SIC/TIBC) × 100], the RBC indices (mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], and, especially, mean corpuscular...
volume [MCV], as well as RBC distribution width [RDW]), and serum ferritin concentration. Examination of a bone marrow aspirate stained with Prussian blue to determine the presence or absence of iron is regarded generally as the “gold standard” for the assessment of storage iron, especially in hospitalized patients.19,25 Unfortunately, this examination is time consuming and expensive, costing as much as $350, compared with the cost ($50) and ease of performing a ferritin determination.26,27 An increased serum ferritin concentration, an increased erythrocyte sedimentation rate (ESR), and marrow iron staining results indicating plentiful iron stores distinguish people with ACD from those with IDA Table I.

Because the serum ferritin concentration can be more conveniently and cost-effectively measured than stainable marrow iron and more than 97% of patients with IDA and no stainable marrow iron have serum ferritin concentrations less than 12 ng/mL (12 µg/L),19,23 ferritin concentration provides a useful screening test for IDA. The principal drawback of serum ferritin concentration to identify IDA is that normal levels may be seen despite iron deficiency in many clinical disorders (eg, chronic infection or inflammation, liver disease, malignant neoplasm) that have a higher prevalence in outpatient clinic and hospitalized patients.18,23,25 Therefore, in this group of anemic patients, it has been suggested that ferritin values below 20 ng/mL (20 µg/L) can be considered diagnostic of iron deficiency.23 By using a ferritin-based biochemical classification of IDA and ACD, we compared TfR with more traditional indicators of iron status for discriminating between IDA and ACD in a group of outpatient clinic and hospitalized patients.

Table I
Classic Distinguishing Features of IDA and ACD*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Iron Deficiency Anemia</th>
<th>Anemia of Chronic Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidemiology</td>
<td>Most common overall</td>
<td>Most common in hospitalized patients</td>
</tr>
<tr>
<td>Type of anemia</td>
<td>Normocytic-normochromic (early stage); Normocytic-normochromic (later stage)</td>
<td>Normocytic-normochromic (60%-70% of cases); microcytic-hypochromic (30%-40% of cases)</td>
</tr>
<tr>
<td>Principal lesion</td>
<td>Anemia secondary to a lack of adequate iron</td>
<td>Suppression of erythropoiesis by mediators of an inflammatory response</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate</td>
<td>↔</td>
<td>↑</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>&lt;31-32 (0.31-0.32)</td>
<td>28-32 (0.28-0.32)</td>
</tr>
<tr>
<td>Mean corpuscular volume</td>
<td>↓</td>
<td>↔</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin</td>
<td>↓</td>
<td>↔</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration</td>
<td>↓ (&gt;14%)</td>
<td>↔ to ↑</td>
</tr>
<tr>
<td>RBC distribution width†</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Serum iron concentration</td>
<td>↓</td>
<td>↔ to ↑</td>
</tr>
<tr>
<td>Total iron binding capacity</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Transferrin saturation (%)†</td>
<td>↓ (&lt;10-15)</td>
<td>↓</td>
</tr>
<tr>
<td>Ferritin concentration, ng/mL (µg/L)†</td>
<td>↓ (&lt;10 [&lt;10])</td>
<td>↑</td>
</tr>
<tr>
<td>Free erythrocyte protoporphyrin concentration</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Stainable marrow iron</td>
<td>Absent</td>
<td>Plentiful</td>
</tr>
</tbody>
</table>

†, decreased; †, increased; ↔, within normal limits.
* Adapted from Cook and Skikne,23 Worwood,24 Duffy,25 and Allen et al.29
† Value(s) in parentheses are typical for this variable.

Materials and Methods

Subjects

We selected randomly 134 serum specimens submitted to the chemistry laboratory of Parkland Memorial Hospital, Dallas, TX, for iron studies, including SIC, TIBC, %TS, and ferritin determinations. Parkland Memorial Hospital is a 1000-bed, tertiary care, county hospital affiliated with The University of Texas Southwestern Medical School, Dallas. SIC, TIBC, and ferritin concentrations were determined using the ferrozine method (SIC and TIBC) in the Paramax chemistry analyzer (Santa Ana, CA) and the immunochemiluminometric assay (ICMA) method (ferritin) in the ACS:180 (Bayer, Walpole, MA) immunoassay analyzer. The %TS was calculated using the formula:

\[
%TS = (\text{SIC}/\text{TIBC}) \times 100
\]

Subsequently, we obtained the results for a CBC count, including values for hemoglobin, hematocrit, RBC count, and RBC indices (MCV, MCH, MCHC, RDW), performed on an EDTA–whole blood sample collected at the same time the whole blood sample for iron studies was obtained. In addition, we validated pregnancy status by medical chart review. Only serum samples from men and nonpregnant women with anemia, defined as a total hemoglobin concentration less than 12.0 g/dL (<120 g/L) in women and less than 13.0 g/dL (<130 g/L) in men and an SIC less than the sex-specific lower limit of the reference range for iron in our laboratory (women, 260 µg/L; men, 760 µg/L), were stored at –70°C for Tfr testing. Samples were stored for no longer than 2 weeks and thawed only once just before Tfr analysis.
Assignment of Subjects to an IDA or ACD Group Based on Laboratory Criteria

Men and nonpregnant women with anemia and a serum ferritin concentration of less than 20 ng/mL (<20 µg/L) were assigned to the IDA group, while those with anemia and a ferritin concentration greater than 1.5 times the sex-specific upper limit of the normal reference range (ie, for women, >240 ng/mL [>240 µg/L]; for men, >375 ng/mL [>375 µg/L]) were assigned to the ACD group. Among our initial 134 subjects, 41 (28 women, 13 men) met the criteria for assignment to the IDA group, while 31 (12 women, 19 men) met the criteria for assignment to the ACD group. The age (mean ± SD) and race distribution among those assigned to our IDA and ACD groups were as follows: IDA group, 47.5 ± 15.5 years; white, 8; black, 14; Hispanic, 18; and Asian, 1; ACD group, 51.0 ± 11.9 years; white, 9; black, 17; and Hispanic, 5. There was no significant difference in the ages between these 2 groups (P = .2980). We performed TfR testing on serum samples from these 72 patients.

TfR Assay

Serum levels of TfR were quantified by enzyme-linked immunosorbent assay according to the manufacturer’s instructions, using kits purchased from R&D Systems (Minneapolis, MN), an ImmunoWash 1575 (Bio-Rad Laboratories, Hercules, CA) for the microtiter plate washing step, and a Microplate Reader 550 (Bio-Rad Laboratories) to read the absorbance in each microtiter plate well at a wavelength of 450 nm without wavelength correction. Patients’ serum samples and quality control serum samples supplied with each kit were tested in duplicate in 5 runs over 5 days with up to 35 samples in each run. The analytic performance characteristics of the R&D TfR assay have been validated, including the reference interval for healthy, nonblack individuals residing in locations at low altitude (8.8-28.1 nmol/L).

Statistical Analyses

All statistical analyses, including least-squares linear regression, 2-tailed t-test (unpaired), and receiver operating characteristic (ROC) curve analysis, were performed using MedCalc software (Mariakerke, Belgium). ROC analysis included calculation of the area under the curve (AUC). AUC values provide a measure of the diagnostic accuracy (ie, sensitivity and specificity) of a test with values from 0.5 to 0.7 representing low accuracy; values from 0.7 to 0.9 representing tests that are useful for some purposes; and values greater than 0.9 representing tests with high diagnostic accuracy. When making comparisons between data, we used a P value of less than .05 as the indicator of statistical significance.

Results

Discriminating Between IDA and ACD using TfR and Indicators of Iron Status and RBC Size

The relationship between TfR and ferritin concentrations in serum samples from patients belonging to the IDA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Reference Interval</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron concentration, µg/L</td>
<td>IDA 153 ± 79</td>
<td>ACD 167 ± 93</td>
<td>F, 260-1,700; M, 760-1,980</td>
</tr>
<tr>
<td>TIBC, mg/L</td>
<td>4.32 ± 0.84</td>
<td>1.98 ± 0.55</td>
<td>2.62-4.74</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>3.7 ± 2.2</td>
<td>9.0 ± 4.5</td>
<td>20-50</td>
</tr>
<tr>
<td>Ferritin concentration, ng/mL (µg/L)</td>
<td>6.7 ± 4.4 (6.7 ± 4.4)</td>
<td>1,052.0 ± 970.6</td>
<td>F, 12-160 (12-160); M, 18-290 (18-250)</td>
</tr>
<tr>
<td>RBC count, x 10^6/µL (x 10^12/L)</td>
<td>3.8 ± 0.7 (3.8 ± 0.7)</td>
<td>3.2 ± 0.6 (3.2 ± 0.6)</td>
<td>F, 3.8-5.5 (3.8-5.5); M, 4.3-6.2 (4.3-6.2)</td>
</tr>
<tr>
<td>Hemoglobin concentration, g/dL (g/L)</td>
<td>8.8 ± 2.0 (88 ± 20)</td>
<td>9.5 ± 1.7 (95 ± 17)</td>
<td>F, 12.0-15.2 (120-152); M, 13.2-15.2 (132-152)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>27.4 ± 5.8 (0.27 ± 0.6)</td>
<td>28.3 ± 5.0 (0.28 ± 0.05)</td>
<td>F, 37.4-46.0 (37.4-46.0); M, 40.6 (40.6-40.6)</td>
</tr>
<tr>
<td>Mean corpuscular volume, µm³ (fL)</td>
<td>72.6 ± 9.8 (72.6 ± 9.8)</td>
<td>875.7 ± 78 (875 ± 78)</td>
<td>F, 79-101 (79-101); M, 82-105 (82-105)</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin, pg</td>
<td>23.3 ± 3.9</td>
<td>29.5 ± 2.9</td>
<td>F, 27-33; M, 28-34</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration, g/dL (g/L)</td>
<td>32.0 ± 13 (320 ± 13)</td>
<td>33.7 ± 0.7 (337 ± 7)</td>
<td>31-35 (310-350)</td>
</tr>
</tbody>
</table>

ACD, anemia of chronic disease; IDA, iron deficiency anemia.

* Data are given as mean ± SD unless otherwise indicated.

* IDA vs ACD group by unpaired Student t-test analysis.
The TfR concentration was increased (mean ± SD, 67.0 ± 32.3 nmol/L) in all patients except 2 in the IDA group (39/41 [95%]) and was normal (22.5 ± 9.0 nmol/L) for more than 75% of the patients (24/31 [77%]) in the ACD group. ROC curve analysis of TfR data and selected indicators of iron status (SIC, TIBC, %TS) and RBC size (MCV, RDW) revealed that the AUC values for these parameters decreased in the following order: 0.993 (AUC TIBC) > 0.958 (AUC TfR) > 0.873 (AUC MCV) > 0.834 (AUC %TS) = 0.834 (AUC RDW) > 0.535 (AUC SIC).

The AUC values for TIBC and TfR, however, were not significantly different (Table 3). Thus, TIBC and TfR measurements provided the highest and similar discriminatory power for distinguishing between our patients with IDA and those with ACD. Optimal cutoff values, diagnostic accuracy, and predictive value parameters for all indices are also shown in Table 3.

**Table 3**

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC</th>
<th>P</th>
<th>Cutoff</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIBC</td>
<td>0.993</td>
<td>.164</td>
<td>3.15 mg/L</td>
<td>92.7</td>
<td>100.0</td>
<td>100.0</td>
<td>91.2</td>
</tr>
<tr>
<td>TfR</td>
<td>0.958</td>
<td>.045</td>
<td>29.5 nmol/L</td>
<td>92.7</td>
<td>83.9</td>
<td>88.4</td>
<td>89.7</td>
</tr>
<tr>
<td>MCV</td>
<td>0.873</td>
<td>.501</td>
<td>79.8 µm³ (79.8 fl)</td>
<td>80.5</td>
<td>83.9</td>
<td>86.8</td>
<td>76.5</td>
</tr>
<tr>
<td>%TS</td>
<td>0.834</td>
<td>.996</td>
<td>6%</td>
<td>92.7</td>
<td>61.3</td>
<td>76.0</td>
<td>86.4</td>
</tr>
<tr>
<td>RDW</td>
<td>0.834</td>
<td>&lt;.001</td>
<td>14.8%</td>
<td>75.6</td>
<td>77.4</td>
<td>81.6</td>
<td>70.6</td>
</tr>
<tr>
<td>SIC</td>
<td>0.535</td>
<td></td>
<td>140 µg/L</td>
<td>51.2</td>
<td>64.5</td>
<td>65.6</td>
<td>50.0</td>
</tr>
</tbody>
</table>

ACD, anemia of chronic disease; AUC, area under the curve; IDA, iron deficiency anemia; MCV, mean corpuscular volume; NPV, negative predictive value; PPV, positive predictive value; RDW, RBC distribution width; SIC, serum iron concentration; TfR, transferrin receptor; TIBC, total iron binding capacity; %TS, percent transferrin saturation.

* AUC for TIBC vs TfR.
† AUC for TfR vs MCV.
‡ AUC for MCV vs %TS.
§ AUC for %TS vs RDW.
|| AUC for RDW vs SIC.

**Discussion**

In the present study, we examined the usefulness of the soluble TfR concentration for distinguishing IDA from ACD and compared it with traditional indices of iron status. The assignment of patients to IDA and ACD groups was made on
Having IDA or ACD. For example, Carriaga et al,\(^5\) in their clinical value (ie, area under the curve = 0.500). The diagonal line represents the ROC curve for a test with no discrimination capacity (MCV), percent transferrin saturation (% TS), RBC distribution width (RDW), and serum iron concentration (SIC). The diagonal line represents the ROC curve for a test with no clinical value (ie, area under the curve = 0.500).

Among the tests we evaluated for discriminating between IDA and ACD in nonpregnant subjects, ROC curve analysis indicated that the TIBC and the TfR concentration provided the highest diagnostic accuracy for discriminating between individuals in these groups, and the difference in the discriminatory power of these 2 analytes was not significant (Figure 3 and Table 3). Compared with TIBC and TfR, MCV, %TS, RDW, and especially SIC were significantly poorer discriminators between IDA and ACD. At the cutoff value for TIBC (3.15 mg/L) at which diagnostic accuracy was highest, the positive and negative predictive values of TIBC for discriminating between IDA and ACD were 100% and 91%, respectively. By contrast, at cutoff values for TfR at which diagnostic accuracy was highest, the positive and negative predictive values of TfR were both approximately 90%. In addition, the cutoff value for TfR (ie, 29.5 nmol/L) at which diagnostic accuracy was highest (Table 3) was close to the upper limit of the reference interval for this assay for healthy nonblack subjects (28.1 nmol/L) and black subjects (29.9 nmol/L) residing at low altitude. Although age-, sex-, race-, and altitude-related changes are either restricted to blacks or are additive (blacks and healthy nonblack subjects), age-, sex-, race-, and altitude-related changes in TfR concentration occur, age- and sex-related changes are absent in adults (older than 20 years),\(^33\) while race- and altitude-related changes in TfR concentration are either restricted to blacks or are additive (blacks residing at low or high altitude) and independently cause approximately only a 9% increase in the TfR concentration.\(^29\)

Various laboratory indicators of iron status have been shown to correlate to varying degrees.\(^34\) Although we evaluated these relationships (data not shown), Hastka et al\(^34\) suggested that such correlations are not helpful for studying the diagnostic value of a laboratory test in IDA. However, integration of values for these various indicators is useful for conceptualizing progressive stages of iron deficiency. Hastka et al\(^34\) provided a useful figure that summarizes the diagnostic sensitivity of various markers in 3 stages of ID. We modified their figure to include TIBC and TfR.

In our study, ferritin concentrations of less than 20 ng/mL (<20 µg/L) or more than 1.5 times the upper limit of normal were effective for identifying groups of individuals likely to have IDA or ACD, respectively (Figure 1). Values for several of the other indicators that we used to classify individuals in our study as having IDA or ACD were consistent with the characteristic differences between these indicators observed typically in people with histologically proven IDA or ACD (Tables 1 and 2). Most notably, values for TIBC and RDW were significantly lower, while values for ferritin and MCV were significantly higher in the ACD group than in those in the IDA group (Table 2). In addition, TfR values were significantly higher in the ACD group than in the IDA group (Figure 2).

At a ferritin cutoff value of 12 ng/mL (12 µg/L), Mast et al\(^34\) demonstrated that ferritin had excellent specificity (98%) but poor sensitivity (20%) for identifying IDA among 58 individuals whose bone marrow samples were stained for the presence of iron, while at a cutoff value of 30 ng/mL (30 µg/L), ferritin was almost (ie, sensitivity, 100%; specificity, 98%) a perfect test (ie, sensitivity = specificity = 100%).
clinical practice. One of the more interesting applications of soluble TfR measurements is the detection of athletes suspected of “doping” with erythropoietin. Doping with erythropoietin has been shown to result in a significant increase in soluble TfR concentration.

Our data, when coupled with the data published previously by Mast et al and others, suggest that the principal role of TfR in clinical practice lies currently in complementing, rather than replacing, the more traditional laboratory tests of iron status to identify iron deficiency, especially when results of these tests are equivocal. This seems especially true when one considers that there is no single “best” test of iron deficiency, and in the clinical setting, iron deficiency must first be detected before its severity can be graded and appropriate therapy instituted.

Previous studies have suggested that serum TfR measurement is a useful laboratory parameter for the distinction of IDA from ACD. While our data demonstrate good discriminatory power of serum TfR in this differential diagnosis, we believe it offers little, if any, advantage over conventional laboratory indicators of iron status, particularly TfBC, for this purpose. We recognize, however, that we evaluated only cases of overt IDA and ACD. The serum TfR may be of value for determining whether a low or borderline low hemoglobin concentration is due to the suppression of erythropoiesis by mediators (eg, interleukin-1 and tumor necrosis factor alpha) of an inflammatory response (normal TfR concentration) as occurs in ACD or to an insufficient quantity of iron when serum ferritin levels are intermediate between those that are diagnostic of IDA or ACD. Moreover, it also may be of value for the evaluation of patients with concomitant IDA and ACD. Ferritin levels may be within normal limits in such patients, while TfR is typically depressed. Measurement of serum soluble TfR levels is effective for evaluating the severity of iron deficiency in these patients because the expression of TfR by cells of the reticuloendothelial system is directly proportional to cellular iron demand, and the serum level of soluble TfR is directly proportional to the total amount of cellular TfRs.

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References


