Association between transferrin receptor-ferritin index and conventional measures of iron responsiveness in hemodialysis patients

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Running title: Transferrin receptor-ferritin index in hemodialysis patients
ABSTRACT

**Background:** The aim of this study was to appraise the diagnostic power of the transferrin receptor-ferritin (TfR-F) index for identification of iron responsiveness in chronic hemodialysis (CHD) patients compared with the routine markers recommended by the current US and European guidelines. **Methods:** Initially, 121 CHD patients with a serum ferritin level of <800 μg/L and on recombinant erythropoietin (rHuEPO) therapy for >6 months were enrolled for intravenous iron (IVFE) supplementation (100 mg iron polymaltose three times a week for 4 weeks, then 100 mg every 2 weeks for 5 months). Routine iron tests (i.e. serum ferritin and transferrin saturation [TSAT]), TfR-F index calculated by the ratio of soluble TfR to log ferritin level, hematocrit, hemoglobin, RBC count and serum high sensitive C-reactive protein were examined at baselines. Hematocrit and hemoglobin were then followed every 2 weeks during the study period. **Results:** A total of 100 patients (52 men and 48 women, mean age of 59 years) completed this study. Fifty-two patients were IVFE responders defined as a rise in hematocrit of >3% and/or a reduction in rHuEPO dose of >30% over the baseline values at the end of the study and 48 non-responders did not fulfill these criteria. Among 52 responders, only 14 patients (27%) could be identified with iron deficiency by means of the routine iron tests (ferritin <100 μg/L and/or TSAT <20%). Thirty-three responders (63%) could be further identified for iron deficiency by using the TfR-F index (>0.6), but 5 (10%) still could not by either method. Analyses by receiver operating characteristic (ROC) curves revealed that a cutoff value of >0.6 for the TfR-F index had the higher sensitivity (90%) for detection of iron deficiency than ferritin at <100 μg/L (29%) and TSAT at <20% (6%). The TfR-F index displayed a greater area under the ROC curve than those of ferritin (P < 0.05) and TSAT (P < 0.001). **Conclusion:** The TfR-F index is superior to the routine tests for predicting the response to IVFE supplementation in
CHD patients. Our study indicates the TfR-F index be a new and surrogate marker to estimate body iron stores and to guide the IVFE therapy for CHD patients.

**INDEX WORDS:** Transferrin receptor-ferritin index; recombinant human erythropoietin; iron deficiency; hemodialysis
INTRODUCTION

Anemia is frequently encountered in patients with end-stage renal disease (ESRD). The advent of recombinant human erythropoietin (rHuEPO) can effectively alleviate or correct ESRD-related anemia and reduce the needs for blood transfusion. Due to accelerated erythropoiesis by rHuEPO, provision of insufficient available iron to erythroblasts will facilitate the development of iron-deficient erythropoiesis. Early detection of iron deficiency is mandatory, since it can be combated with intravenous iron (IVFE) supplementation and thereby reduce the doses of rHuEPO. However, aggressive IVFE therapy must be weighed in the context of iron-associated risks such as hypersensitivity, hemosiderosis, hepatic, cardiovascular and infectious morbidities, and increased oxidative stress.

To maximize the efficacy of rHuEPO therapy and avoid iron toxicity, it is of supreme importance to find a marker for early predicting who will get improvement in erythropoiesis following IVFE supplementation. The conventional markers serum ferritin and transferrin saturation (TSAT) are widely used to indicate iron status in chronic hemodialysis (CHD) patients. However, serum levels of ferritin and transferrin are affected by the status of inflammation and malnutrition, respectively. Moreover, TSAT fluctuates due to a diurnal variation in serum iron. Cutoff levels of ferritin and TSAT for predicting iron-deficient erythropoiesis during rHuEPO therapy still remains debatable.

Iron delivery to erythroblasts is mediated by the interaction of transferrin with cell surface transferrin receptor (TfR), and TfR expression is induced by a decrease in the level of intracellular free iron. Soluble TfR (sTfR) in plasma is a truncated form of the tissue receptor. We and others have shown that elevated sTfR level is a surrogate marker of iron status in CHD patients and not affected by acute phase response as in serum ferritin, TSAT and percent of hypochromic red cells. On the
other hand, the total mass of erythropoiesis has a positive effect on the concentration of sTfR in plasma. Elevated sTfR may reflect hyperplastic erythropoiesis induced by rHuEPO. Therefore, some studies failed to demonstrate sTfR as an iron-deficient index partly due to the confounding effect of rHuEPO-induced erythropoiesis in HD patients.\textsuperscript{17,18}

Cumulative data have demonstrated the TfR-log ferritin (TfR-F) index as a noteworthy tool to differentiate iron deficient anemia from anemia of hyperplastic erythropoiesis, i.e. thalassemia, hemolytic anemia, and myelodysplasia.\textsuperscript{6,19--21} The TfR-F index is superior to sTfR alone for identification of latent iron deficiency in patients with anemia of chronic disease\textsuperscript{6,19} and high C-reactive protein (CRP) levels.\textsuperscript{19,21--23} To the best of our knowledge, there is no study stating whether the TfR-F index is feasible to identify the functional iron deficiency in rHuEPO-treated CHD patients. Bone marrow biopsy is traditionally the gold standard measure of iron stores. Nevertheless, it is invasive and not practical to perform serial follow-up biopsies to monitor the iron status in CHD patients. Accordingly, the aim of this study is to investigate whether the TfR-F index can early detect iron deficiency and predict the responsiveness to IVFE therapy as compared with the conventional iron parameters in CHD patients.

**METHODS**

**Patients and Study Design**

A prospective study was conducted at the dialysis center of the affiliated hospital of National Yang-Ming University, Taipei. Firstly, 121 patients agreed to participate in this study. The inclusion criteria were as follows: on HD treatment for 6 months, on rHuEPO therapy for 6 months, serum ferritin of <800 \( \mu \text{g/L} \), no hematological disorder other than renal anemia, no blood transfusions or iron supplementation in the
preceding 3 months and no inflammatory diseases or infections that might affect the
erthropoietic response to rHuEPO therapy. Twenty-one patients were excluded
during the study due to clinically significant bleeding ($n = 6$), infections ($n = 8$),
treatment modality shift to peritoneal dialysis ($n = 2$) or renal transplantation ($n = 3$),
and poor compliance to IVFE therapy ($n = 2$). Finally, a total of 100 patients (52 men
and 48 women, mean age of 59 years) completed the study. All patients were dialyzed
for 4.0–4.5 h three times a week, using a single-use dialyzer with a 1.5 m$^2$ effective
surface area of cellulose diacetate membrane, blood flow of 300–350 ml/min and
dialysate flow of 500 ml/min. The study was approved by the local medical ethics
committee and informed consent was obtained from each of the patients.

All patients were supplied with 100 mg iron polymaltose (Ferrum Hausmann,
Hausmann Lab. Inc., Switzerland) three times a week for 4 weeks (total dose of 1200
mg elemental iron) and then 100 mg iron polymaltose every 2 weeks for 5 months
(total dose of 1000 mg elemental iron). Iron supply was administered intravenously at
the end of each HD session. Before the first dose was administered, a test dose of 25
mg of iron polymaltose was administered for 30 min to observe any adverse reaction
development. If no adverse reactions occurred, the IVFE supplementation protocol
would then be started. A response to IVFE therapy was defined as a rise in hematocrit
of $\geq 3\%$ (i.e. a rise from 30% to 34%) and/or a reduction in rHuEPO dose of 30% over
the baseline values at the end of the study. Those who did not fulfill the
abovementioned criteria were defined as non-responders. Routine iron tests (i.e. TSAT,
serum iron and ferritin), TfR-F index, hematocrit, hemoglobin, RBC count,
reticulocyte count, serum albumin and high sensitive CRP (hs-CRP) were examined at
baselines. Hematocrit and hemoglobin were then followed every 2 weeks during the
study period. Epoetin $\beta$ (Roche Diagnostics GmbH, Mannheim, Germany) was
administered subcutaneously two or three times a week, and the dose was titrated
biweekly to maintain a target hematocrit value of 32-33%. During the study period, the epoetin β dose could be adjusted by 20% every two weeks. For instance, a 20% reduction in the dose if a hematocrit level >33% was recorded, or a 20% increase in the dose if a hematocrit level had decreased >3% from the baseline levels.

**Laboratory Measurements**

Blood was drawn pre-dialysis after an overnight fast. Hematocrit, hemoglobin, and RBC count were determined with a Coulter counter and reticulocyte was measured by an automated flow cytometer. Serum iron was measured by a calorimetric method (Hitachi 736-60 autoanalyzer; Hitachi, Naka, Japan), total iron binding capacity by the TIBC Microtest (Daiichi, Tokyo, Japan) and ferritin by radioimmunoassy (Incstar, Stillwater, MN, USA). TSAT was calculated by dividing serum iron by TIBC × 100. Serum hs-CRP was quantitatively determined by rate immunotubidimetry using a commercially available kit in conjunction with IMMAGE® immunochemistry system (Beckman Coulter, Fullerton, CA). Serum sTfR concentrations were measured by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA) according to the procedure recommended by the manufacturer. The central 95th percentile of the reference distribution of this assay is 740–2390mg/l in 225 healthy persons described by the manufacturer. The minimum detectable level was <42.3 mg/l (0.5nmol/l). Intra-assay coefficient of variance of the assay for stir ranged from 6 to 9% and inter-assay coefficient of variance ranged from 8 to 10%. To make the test more specific, the TfR-F index was calculated by the ratio of sTfR to log ferritin level, where sTfR level is measured in milligrams per liter and ferritin level in micrograms per liter.

**Statistical Analysis**
Statistical analysis was performed using the computer software Statistical Package of Social Science (SPSS 11.0, 2001; SPSS Inc., Chicago, IL, USA). Data were expressed as means ± SD. Serum ferritin and hs-CRP values were reported as median and inter-quartile range because the data were not normally distributed and positively skewed. Comparisons of data for responders and non-responders were performed using the *t*-test for normally distributed variables or Mann–Whitney U-test for variables with non-normal distribution. Data of more than two groups were analyzed using one-way ANOVA or Kruskall-Wallis test followed by pair-wise multiple comparisons for significance of difference. Pearson’s chi-square test was used for frequency measures. To identify optimal threshold values for predicting iron deficiency, receiver operating characteristics (ROC) curve analysis was performed by computing sensitivity and specificity of the different tests at various cutoff levels. Sensitivity was defined as the percentage of responsive patients who had a positive test, and specificity as the percentage of unresponsive patients who had a negative test.

To identify the independent predictors of iron deficiency, we used the forward stepwise logistic regression analysis with IVFE responder as the dependent variable. Independent variables included age, gender, dialysis vintage, TfR-F index, serum ferritin, TSAT, serum hs-CRP, albumin and intact PTH levels, and Kt/V. The odds ratios were calculated according to the Mantel-Haenszel for the independent variables entered into the model. A *P* value of <0.05 was considered statistically significant.

**RESULTS**

Basal characteristics between IVFE responders (*n* = 52) and non-responders (*n* = 48) were similar with regard to age (58 ± 14 vs 60 ± 14 y), gender distribution (male, 52% vs 52%), HD duration (66 ± 51 vs 54 ± 48 mo), and etiology of renal failure. Baseline serum values of ferritin (175 [78 to 263] vs 410 [296 to 490] μg/L, *P* <0.001)
and hs-CRP (7.0 [3.7 to 20] vs 28.4 [5.3 to 84] mg/L, \( P = 0.004 \)) were significantly lower and TfR-F index (0.82 ± 0.33 vs 0.53 ± 0.11, \( P < 0.001 \)) was higher in the responders than in non-responders. Other parameters such as TSAT (34 ± 10% vs 36 ± 10%), serum levels of albumin (4.0 ± 0.4 vs 3.9 ± 0.3 g/dL) and intact parathyroid hormone (216 ± 239 vs 156 ± 165 ng/L) and Kt/V (1.42 ± 0.30 vs 1.45 ± 0.28) showed no significant differences between the two groups of patients. After 6 months of IVFE therapy, erythropoiesis was significantly enhanced when compared with the baseline values (hemoglobin, 9.6 ± 1.0 to 11.0 ± 1.2 g/dL and hematocrit, 29.4 ± 3.0 to 33.4 ± 3.4 %, \( P < 0.001 \)) in the responders; and the reduction in rHuEPO dose was also noted (100 ± 48 to 63 ± 42 U/Kg/week, \( P < 0.001 \)). In the non-responders, there were no significant changes in erythropoiesis (hemoglobin, 9.6 ± 1.1 to 9.3 ± 1.4 g/dL and hematocrit, 29.6 ± 3.3 to 27.7 ± 3.1 %, \( P > 0.05 \)) at a constant rHuEPO dose (85 ± 40 to 84 ± 34 U/Kg/week, \( P > 0.05 \)) despite 6 months of IVFE supplementation (Figure 1).

Among 52 responders, only 14 patients (27%, Group 1) were identified by the conventional tests for iron deficiency (ferritin <100 μg/L and/or TSAT <20% recommended by the international guidelines).\(^7,8\) As shown in Table 1, mean levels of hemoglobin and hematocrit and mean corpuscular volume were similar to those of the other groups. But serum levels of ferritin, TSAT and hs-CRP were significantly lower compared with the other groups of patients. The TfR-F index clearly identified iron deficiency in these 14 patients with a mean value of 1.20 ± 0.35. By using the TfR-F index, we could identify another 33 (63%, Group 2) of the 52 iron-deficient patients who responded to IVFE therapy. None of the 33 patients could have been identified by means of the routine tests for iron deficiency. In 5 other responders (10%, Group 3), the diagnosis could not be established by means of either method. These 5 patients had higher serum ferritin and lower TfR-F index than those in the patients of Groups
1 and 2 (Table 1). After 6 months of IVFE supplementation, serum ferritin and TSAT significantly increased compared to the baseline values, while the TfR-F index significantly decreased to a mean value of <0.6 in the responders and the patients in Group 1 to 3 (Figure 1).

Forty-eight non-responders (Group 4) had the similar age, gender distribution, HD duration, weekly rHuEPO dose, as well as mean corpuscular volume and mean levels of hemoglobin, hematocrit and reticulocyte count compared with those of the other groups (Table 1). Median ferritin level was the highest in Group 4, and 38 (79%) of these patients had an TfR-F index lower than 0.6, excluding iron deficiency as the cause of anemia. Only 10 non-responders who had raised levels of TfR-F index (0.70 ± 0.11) showed low levels of serum ferritin (174 [88 to 230] μg/L) and TSAT (34 ± 13%). We could not exclude the likelihood of an early stage of iron deficiency in these 10 patients; however, the presence of no response to IVFE therapy seemed not support this postulation. Mean level of serum ferritin modestly increased 6 months following IVFE therapy in the non-responders, and the mean values of TSAT and TfR-F index showed no significant changes (Figure 1). There were no serious adverse events during iron polymaltose treatment in this study. No hospitalization or death related to drug administration was noted. Adverse events probably related to treatment were experienced by 3 patients and included pruritus in 2 responders and abdominal pain and vomiting in 1 non-responder.

We further analyzed the cutoff values of TfR-F index, serum ferritin and TSAT using ROC curves to obtain the sensitivity and specificity in the prediction of iron-deficient anemia. The cutoff values for TfR-F index of >0.6, ferritin of <300 μg/L and TSAT of <30% exhibited the best sensitivity and specificity between the two groups of patients with and without a response to IVFE therapy (Table 2). The TfR-F index had high sensitivity (90%) and specificity (79%) for the diagnosis of iron
deficiency in our CHD patients compared with the low sensitivity (27%) of the routine tests used for the evaluation of iron deficiency. Figure 2 further shows that the TfR-F index displayed a greater area under the ROC curve than that of ferritin \((P < 0.05)\) or TSAT \((P < 0.001)\). The threshold values for detecting iron deficiency were serum ferritin <100 μg/L, TSAT <20% (model 1) or serum ferritin <300 μg/L, TSAT <30% (model 2) and TfR-F index >0.6 in the multiple regression analysis (Table 3). Logistic regression analysis displayed that only the TfR-F index in model 1 and the TfR-F index together with serum ferritin at baseline in model 2 exhibited a significant odds ratio in prediction of response to IVFE supplementation after adjustment for the other variables in the model.

**DISCUSSION**

Iron deficiency is highly prevalent in CHD patients, especially in those treated with rHuEPO. Bone marrow biopsy stained to assess iron is the gold standard for an estimate of iron stores. However, it is impractical to perform serial bone marrow biopsies to monitor iron status in CHD patients.\(^7,8\) Current US guidelines advocate aggressive IVFE therapy in rHuEPO-treated patients to achieve and maintain the target hemoglobin of 11–12 g/dL and hematocrit level of 33–36%.\(^7\) Moreover, the response to IVFE therapy, either to reduce rHuEPO requirements in achieving a hemoglobin target or to increase erythropoietic response at a constant rHuEPO dose, has been thought to be an alternative criterion for iron deficiency in CHD patients.\(^24–27\) However, parenteral iron medications carry a high risk of complications in ESRD patients.\(^4–6\) Thus, to avoid the inadvertent hazards from an unnecessary treatment one should not recommend IVFE therapy without a clear indication, i.e. a definitive diagnosis of iron deficiency. In this study, the safety of
maintenance intravenous iron polymaltose therapy was similar to that of either a single total dose infusion of iron 900 to 3200 mg\(^{28}\) or an intermittent course of iron 1000 mg.\(^{29}\) The observation period, however, is too short to address the issue of increased risk of infection or morbidity and/or mortality from receiving frequent doses of iron polymaltose.

Nowadays, no single test of iron parameters strongly indicates the presence of functional iron deficiency in CHD patients receiving rHuEPO treatment.\(^{9,10}\) Serum iron, TSAT and ferritin levels often conceal the diagnosis of iron deficiency in CHD patients, mainly due to the coexistence of anemia of chronic disease and subclinical inflammation in most patients.\(^{6-10}\) Our data revealed a low level of utility (both sensitivity and specificity <80%) using serum ferritin at a cutoff of <100 \(\mu g/L\) and TSAT <20% for prediction of response to IVFE therapy, which is much worse than that reported by previous studies.\(^9,30,31\) Two tests, serum ferritin at a level of <300 \(\mu g/L\) and TSAT <30%, provided marginal utility in the present study. Accordingly, our research and others\(^2,9,10,27,32\) suggest that the threshold serum ferritin and TSAT levels below which iron depletion is predictably present in a CHD patient are higher than those currently used for the assessment of iron deficiency.\(^7,8\) Functional iron deficiency can occur at TSAT levels approaching 30% and ferritin levels in excess of 300 \(\mu g/L\). However, in our study, the sensitivity of the test increased to 85% but the specificity decreased to 54% using the combination of the 2 indices (ferritin <300 \(\mu g/L\) and/or TSAT <30%). Since the commonly-used iron measures are unreliable in diagnosing this factor, the only way to show functional iron deficiency is to assess the erythroid response to a surplus of iron administration. Therefore, we used this functional approach to define the utility of the Tf\(\text{R}-\text{F}\) index compared with the conventional iron measures in an unselected hemodialysis population.
The TfR-F index represents the total-body iron stores and the availability of iron for erythropoiesis.\textsuperscript{12-14,22} Cumulative data have shown that this index is superior to other parameters in the diagnosis of iron deficiency in some anemic patient groups.\textsuperscript{6,20,21,23} Therefore, it is suggested that the TfR-F index be a surrogate guide to identify iron deficiency among CHD patients with or without increased erythropoiesis. Our findings show an additional 63\% with patients of functional iron deficiency identified by using the TfR-F index at a cutoff value of >0.6. None of these patients can be recognized by the routine iron metabolism indices. The TfR-F index at a value of >0.6 has the greatest utility of the tests studied (Table 2). Moreover, stepwise multivariate regression analysis illustrates a major, independent effect of the TfR-F index in predicting the response to IVFE supplementation (Table 3). Our data substantiate that the TfR-F index improves the diagnostic value of the routine tests for latent iron depletion. Studies of reticulocyte hemoglobin content at a level of <28 pg in the diagnosis of functional iron deficiency by Mittman et al.\textsuperscript{33} and our group\textsuperscript{34} showed sensitivity/specificity numbers (78\%/71\% and 78\%/87\%) close to those (90\%/79\%) for the TfR-F index reported in the present study. sTfR alone at a level of >1.5 mg/L by Tessitore et al.\textsuperscript{31} showed a sensitivity/specificity of 81\%/71\%, which is mildly inferior to the TfR-F index. To our knowledge, this is the first study to demonstrate the accuracy of diagnosis for functional iron deficiency by means of the TfR-F index in chronic HD patients.

Cutoff values of the TfR-F index for iron deficiency varies from 0.8 to 1.5 in different series.\textsuperscript{6,20,21,23} Reasons of different study populations, bias in sample size, presence of anemia of chronic disease and inflammation or not, and different diagnostic criteria for iron deficiency may possibly account for the variance in TfR-F threshold values. Some investigators propose an TfR-F ratio of >1.5 to discriminate
iron-deficient anemia from hyperplastic erythropoiesis and anemia in the elderly,
and a ratio of >1 from anemia of chronic disease. Others further observed that iron-deficient erythropoiesis was indicated by an TfR-F index value >1.5 in patients without acute phase response, but the corresponding value in those with acute phase response was >0.8. Accordingly, these findings do not concur that only one fixed TfR-F index value was used to distinguish iron-deficient anemia from anemia of chronic disease and the coexistence of functional iron deficiency in anemia of chronic disease.

Multiple comorbidities and subclinical inflammation with elevated levels of CRP are common in CHD patients. That is, anemia of chronic disease is highly linked with anemia in ESRD patients. Serum ferritin measurement is of value as an indirect indicator of total body iron stores in CHD patients. However, ferritin levels are affected by inflammation. Investigators have observed that CHD patients with inflammation who present with higher ferritin levels in serum are more likely to suffer from severe anemia. Our data corroborate these findings that a trend in the increase of serum ferritin level from Group 1 to Group 4 was paralleled by an increase in hs-CRP (Table 1). Since sTfR is unaffected in acute phase response, the increase in serum ferritin decreases the TfR-F index in our CHD patients. Therefore, it is plausible to apply the lower TfR-F index value for diagnosis of iron deficiency in CHD patients receiving rHuEPO therapy. Actually, the cutoff values at >0.8–1.5 had low sensitivity for identification of iron deficiency in the present study. These higher cutoff values provide limited diagnostic power in the early detection of iron avid patients, similarly as the routine iron tests (Table 2). Furthermore, investigators showed a close correlation among three methods used to determine sTfR levels. Since the TfR test has been validated and standardized among manufacturers, the cutoff values reported here might be applicable to use other TfR test kits. Accordingly,
we conclude that the TfR-F index (>0.6) provides an attractive tool for accurately obtaining a diagnosis of functional iron deficiency and making an accurate therapeutic judgment on IVFE supplementation in CHD patients.
REFERENCES


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Table 1. Characteristics and baseline laboratory data of chronic hemodialysis patients recruited in this study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IVFE Responder&lt;sup&gt;b&lt;/sup&gt; (n = 52)</th>
<th>IVFE Non-responder&lt;sup&gt;b&lt;/sup&gt; (n = 48)</th>
<th>P value</th>
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<tr>
<td>Patients No. in each subgroup</td>
<td>14</td>
<td>33</td>
<td>5</td>
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<tr>
<td>Demographics</td>
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<tr>
<td>Age, years</td>
<td>62 ±11</td>
<td>56 ±15</td>
<td>58 ±16</td>
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<tr>
<td>Male/female</td>
<td>11/3</td>
<td>15/18</td>
<td>1/4</td>
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<tr>
<td>Hemodialysis duration, mo</td>
<td>24 ±13</td>
<td>88 ±70</td>
<td>30 ±18</td>
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<td>Epoetin β dose, U/kg/wk</td>
<td>82 ±57</td>
<td>106 ±42</td>
<td>122 ±44</td>
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<td>Laboratory data</td>
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<tr>
<td>Albumin, g/dL</td>
<td>3.9 ±0.3</td>
<td>4.0 ±0.4</td>
<td>4.0 ±0.5</td>
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<tr>
<td>hs-C-reactive protein, mg/L</td>
<td>4.2 (3.7 to 44)</td>
<td>4.7 (3.5 to 20)</td>
<td>16.5 (7 to 38)&lt;sup&gt;i&lt;/sup&gt;</td>
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<tr>
<td>Kt/V</td>
<td>1.2 ±0.3</td>
<td>1.4 ±0.3b</td>
<td>1.9 ±0.1&lt;sup&gt;bi&lt;/sup&gt;</td>
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<tr>
<td>Intact parathyroid hormone, ng/L</td>
<td>209 ±172</td>
<td>232 ±274</td>
<td>124 ±118</td>
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<tr>
<td>Reticulocyte count, %</td>
<td>1.7 ±0.7</td>
<td>1.9 ±0.6</td>
<td>1.9 ±0.7</td>
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<td>Hemoglobin, g/dL</td>
<td>9.7 ±1.0</td>
<td>9.7 ±1.0</td>
<td>9.2 ±1.0</td>
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<td>Hematocrit, %</td>
<td>29.5 ±3.5</td>
<td>29.7 ±2.9</td>
<td>27.6 ±3.0</td>
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<td>Mean corpuscular volume, fl</td>
<td>86 ±7</td>
<td>91 ±9</td>
<td>89 ±4</td>
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<td>Iron metabolism indices</td>
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<tr>
<td>Serum ferritin, μg/L</td>
<td>44 (34 to 61)</td>
<td>213 (165 to 263)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>359 (327 to 435)&lt;sup&gt;bi&lt;/sup&gt;</td>
</tr>
<tr>
<td>TSAT, %</td>
<td>28 ±9</td>
<td>37 ±10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32 ±9&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Serum iron, μg/dL</td>
<td>77 ±33</td>
<td>84 ±19</td>
<td>65 ±20</td>
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<tr>
<td>TfR-F index</td>
<td>1.20 ±0.35</td>
<td>0.78 ±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53 ±0.03&lt;sup&gt;bi&lt;/sup&gt;</td>
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</table>

<sup>a</sup> IVFE, intravenous iron; TSAT, transferrin saturation; TfR-F index, transferrin receptor–ferritin index.

<sup>b</sup> IVFE response means iron deficiency by definition of an increase of hematocrit >3% and/or a

hs-C-reactive protein and serum ferritin values are reported as medians and interquartile range because the data are not normally distributed; other continuous variables are reported as means ± SD.

<sup>c</sup> IVFE, intravenous iron; TSAT, transferrin saturation; TfR-F index, transferrin receptor–ferritin index.
reduction of rHuEPO dose >30%; IVFE non-response means iron repletion.

d Serum ferritin <100 μg/L and/or TSAT <20% for the diagnosis of iron deficiency by the current US and European guidelines.7,8

d One-way ANOVA.

e Pearson x² tests.

f Mann-Whitney U test.

# Kruskal-Wallis test.

h P <0.05 versus Group 1.

i P <0.05 versus Group 2.

j P <0.05 versus Group 3.

NOTE. To convert serum albumin in g/dL to g/L, multiply by 10; hemoglobin in g/dL to g/L, multiply by 10; serum iron μg/dL to μmol/L, multiply by 0.179.
Table 2. Diagnostic properties of different thresholds of serum ferritin, TSAT and TfR-F index for predicting iron-deficient anemia in hemodialysis patients

<table>
<thead>
<tr>
<th>Index</th>
<th>Cutoff values</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (μg/L)</td>
<td>&lt;100</td>
<td>29</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>&lt;200</td>
<td>58</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>&lt;300</td>
<td>83</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>&lt;400</td>
<td>92</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>&lt;500</td>
<td>99</td>
<td>35</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>&lt;20</td>
<td>6</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>&lt;30</td>
<td>58</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>&lt;40</td>
<td>65</td>
<td>33</td>
</tr>
<tr>
<td>Routine lab tests(^b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin (μg/L)</td>
<td>&lt;100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and/or TSAT (%)</td>
<td>&lt;20</td>
<td>27</td>
<td>91</td>
</tr>
<tr>
<td>Ferritin (μg/L)</td>
<td>&lt;300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and/or TSAT (%)</td>
<td>&lt;30</td>
<td>85</td>
<td>54</td>
</tr>
<tr>
<td>TfR-F index</td>
<td>&gt;0.6(^c)</td>
<td>90</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>&gt;0.8</td>
<td>33</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>&gt;1.0</td>
<td>27</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>&gt;1.5</td>
<td>4</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^a\) TSAT, transferrin saturation; TfR-F index, transferrin receptor–ferritin index.

\(^b\) Serum ferritin <100 μg/L and/or TSAT <20% for the diagnosis of iron deficiency by the current US and European guidelines.\(^7,8\)

\(^c\) The optimal sensitivity and specificity in each group.
Table 3. Independent predictors of response to intravenous iron medications in hemodialysis patients by using logistic regression analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Odds ratio</th>
<th>95% C.I.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TfR-F index</td>
<td>60.7</td>
<td>12.6 to 231.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TfR-F index</td>
<td>40.7</td>
<td>11.5 to 134.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>11.2</td>
<td>4.4 to 28.4</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

1 Cutoff points: serum ferritin at 100 μg/l, TSAT at 20%, and TfR–F index at 0.6 in model 1; serum ferritin at <300 μg/l, TSAT at <30%, and TfR-F index at >0.6 in model 2.

2 Independent variables included age, gender, dialysis vintage, TfR-F index, ferritin, TSAT, serum levels of high sensitive C-reactive protein, albumin and intact parathyroid hormone, and Kt/V.

Abbreviations are: TfR-F index, transferrin receptor–ferritin index; C.I., confidence interval.
FIGURE LEGENDS

Figure 1. Changes in the hematocrit level, epoetin β dose, serum ferritin, transferrin saturation (TSAT) and transferrin receptor-ferritin (TfR-F) index following intravenous iron supplementation for 6 months. There were 52 responders (black bars) and 48 non-responders (white bars). Responders were further stratified into 3 groups: Group 1 (hatched bars, n = 14), Group 2 (gray bars, n = 33), and Group 3 (crossed bars, n = 5). Brackets indicate SD. \(^{a}P < 0.05\), \(^{b}P < 0.001\) vs. baselines.

Figure 2. Receiver operating characteristics (ROC) curve analysis of the transferrin receptor–ferritin index, serum ferritin and transferrin saturation (TSAT). Dashed line at 45° indicates no discriminative ability. Symbols are: (——) TfR-F index; (−−−) serum ferritin; and (−··−) TSAT. Comparisons of the area under ROC curve: TfR-F index vs. ferritin, \(P = 0.037\), and TfR-F index vs. TSAT, \(P < 0.001\).