

The Evaluation of Iron Status in Hemodialysis Patients¹

Steven Fishbane,² Edward A. Kowalski, Louis J. Imbriano, and John K. Maesaka

S. Fishbane, E.A. Kowalski, L.J. Imbriano, J.K. Maesaka, Winthrop-University Hospital, Mineola, NY, and State University of New York at Stony Brook School of Medicine, Stony Brook, NY

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ABSTRACT

Effective treatment of anemia in hemodialysis patients requires ongoing monitoring of iron status. The purpose of this study was to determine levels of commonly used iron indices predictive of iron deficiency in this population. Forty-seven patients with baseline serum ferritin levels <600 ng/mL were treated with intravenous iron dextran (INFeD; Schein Pharmaceutical Inc., Florham Park, NJ), 1000 mg over ten hemodialysis treatments. Patients whose hematocrit value increased by 5% or who had a 10% decrease in their erythropoietin dose by 2 months were classified as having iron deficiency ($N = 31$; 66%). All other subjects were classified as having adequate iron ($N = 16$; 34%). There was no statistically significant difference in baseline serum ferritin, transferrin saturation, mean cell volume, mean cell hemoglobin content, or red cell distribution width between the two groups. Receiver operator curves demonstrated that none of the iron indices had a high level of utility (both sensitivity and specificity >80%). Two tests had marginal utility, serum ferritin at a level of <150 ng/mL, and transferrin saturation <21%. It was concluded that because of the tests' marginal utility, they should only be interpreted in the context of the patient's underlying erythropoietin responsiveness. In patients who are responsive to erythropoietin, a transferrin saturation value <18% or serum ferritin level <100 ng/mL should be used to indicate inadequate iron. When erythropoietin resistance is present, transferrin saturation of <27% or serum ferritin <300 ng/mL should be used to guide iron management.

Key Words: Anemia, iron deficiency, ferritin, transferrin saturation, erythropoietin

The treatment of anemia in hemodialysis patients is frequently blunted by the presence of insufficient iron (1). One factor that has hindered iron man-

agement in this population has been the difficulty in using common laboratory tests to detect iron deficiency. The lack of a clear definition of what constitutes abnormal indices has contributed to this problem. The two tests used most commonly for this purpose in hemodialysis patients, despite a dearth of data to support their value, have been those for serum ferritin and transferrin saturation. The serum ferritin level correlates to some degree with whole body iron storage (iron is stored in reticuloendothelial cells in the liver, spleen, and bone marrow, and in hepatocytes). The transferrin saturation value is a ratio of serum iron to the total iron binding capacity. Although less than 0.1% of iron in the body is found in this pool, the ratio's value derives from the fact that it reflects iron readily available for erythropoiesis. In nonuremic patients, a transferrin saturation value of less than 15% is somewhat predictive of iron deficiency (2). In hemodialysis patients, a value of <20% has been suggested as an indication for intravenous iron therapy (3-5). The serum ferritin level is normally 8 to 30 ng/mL in nonuremic populations (6). In hemodialysis patients, several values from 50 to 600 ng/mL have been suggested as indicating iron deficiency (7-9). Many other tests, including the mean cell volume, mean cell hemoglobin content, and red cell distribution width have also been used for the assessment of iron status (2). In a previous study, we used a functional approach to evaluate these tests in a population of hemodialysis patients with a high pretest probability of iron deficiency (10). To accurately define the performance of diagnostic tests as they would be used in clinical practice, unselected populations need to be studied. The purpose of our current study, therefore, was to evaluate noninvasive tests for the detection of iron deficiency in a general hemodialysis population.

METHODS

Patient Selection

The Winthrop-University Hospital Dialysis Center has 214 hemodialysis patients. Patients were eligible for this study if their serum ferritin level was less than 600 ng/mL. Patients were excluded if they had experienced recent bleeding episodes, if they had not been on a stable recombinant human erythropoietin (rHuEPO) dose for at least 2 months, if they had a history of intolerance to intravenous iron dextran, if they had a life expectancy that was expected to be less than 6 months, if they had any hematologic disease impacting on erythropoiesis (other than ESRD), or if they would not give consent.

Protocol

All patients were given a course of intravenous iron dextran (INFeD; Schein Pharmaceutical Inc., Florham Park, NJ), 1000 mg divided over ten hemodialysis treatments, after informed consent has been obtained from each of them. Each patient's hematocrit value and rHuEPO dose were recorded at base-

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² Correspondence to Dr. S. Fishbane, 222 Station Plaza North, Suite 510, Mineola, NY 11501.

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line, and then every 2 wk for 3 months. Diagnostic tests for iron deficiency measured at baseline included the mean cell volume (MCV), mean cell hemoglobin content (MCHC), red cell distribution width (RDW), serum ferritin, and transferrin saturation.

The dose of rHuEPO was adjusted according to the following protocol: If the hematocrit value was <30%, the rHuEPO dose was increased by 25%. If the hematocrit value was >34%, the dose was decreased by 25%. The study period was 2 months. The rHuEPO dose had to have been stable for 4 wk before the end of study in an individual patient. If it had not been, then the period of follow-up was extended up to 3 months until this criterion was achieved. A stable improvement in erythropoiesis was defined at study completion as an increase in hematocrit value of at least 5% (e.g., from 30% to 35%), or a decrease in the rHuEPO dose of at least 10%. Patients meeting this definition were defined as having iron deficiency at study entry. All other patients were considered to have had adequate iron. Patients were withdrawn from study if they had a bleeding episode requiring transfusion, any intercurrent illness requiring hospitalization, or were transplanted during the study period.

Statistical Analysis

The *t* test was used to compare means between the two groups. Results are expressed as the mean \pm SD. A statistically significant result was defined as a *P* value of <0.05.

Epidemiologic assessments included calculations of sensitivity, specificity, and construction of receiver operator curves (ROC). Sensitivity is defined as the percentage of patients with the disease who test positive with the diagnostic test of interest. Specificity is the percentage of patients who do not have the disease who test negative.

ROC were constructed to display graphically the diagnostic utility for each test. The first step in this process is the calculation of sensitivity and specificity at varying screening values for each diagnostic test, i.e., transferrin saturation of 15%, 18%, 21%, etc. A plot of sensitivity (on the y-axis) against 100 minus specificity (on the x-axis), shows the curve of an ideal test to pass through a point near the upper left hand corner (one that maximizes sensitivity and specificity).

RESULTS

Fifty patients were studied. Three were withdrawn during the study period, one for an episode of gastrointestinal bleeding requiring blood transfusion, and two after renal transplantation. The characteristics of the 47 remaining patients are displayed in Table 1.

Thirty-one of 47 patients (66%) had a positive erythropoietic response (as defined in the Methods section) after the intravenous iron course, and were classified as having iron deficiency at baseline (Table 2). The remaining 16 patients (34%) did not have a positive response to the intravenous iron therapy, and were defined as having adequate iron at baseline. One month before baseline, the mean hematocrit value was $30.1 \pm 3.0\%$ in patients subsequently defined as having iron deficiency, compared with $31.2 \pm 2.2\%$ in patients with adequate iron (*P* = not significant [NS]). The mean hematocrit at baseline was $30.3 \pm 3.1\%$ in patients with iron deficiency, compared with $31.7 \pm 4.1\%$ in patients with adequate iron (*P* = NS). The mean rHuEPO dose at baseline in patients with iron

TABLE 1. Patient characteristics

| | |
|----------------------------|----------------|
| N | 47 |
| Age | 53.6 \pm 5.2 |
| Men:Women | 27:20 |
| Race | |
| White | 30 |
| Black | 9 |
| Hispanic | 7 |
| Other | 1 |
| Etiology of ESRD | |
| Diabetes mellitus | 20 |
| Hypertension | 18 |
| Polycystic kidney disease | 4 |
| Chronic glomerulonephritis | 2 |
| Unknown | 3 |
| Years on Dialysis | 2.3 \pm 0.5 |

TABLE 2. Mean hematocrit values and recombinant human erythropoietin (rHuEPO) doses at baseline for the two study groups

| Measure | Inadequate Iron Stores (N = 31) | Adequate Iron Stores (N = 16) | <i>P</i> Value |
|--------------------------------|---------------------------------|-------------------------------|----------------|
| Mean Hematocrit Value (%) | 30.3 \pm 3.1 | 31.7 \pm 4.1 | 0.20 |
| Mean rHuEPO Dose (U/treatment) | 7387 \pm 300 | 6094 \pm 300 | 0.16 |

TABLE 3. Mean baseline values for the iron indices studied in the two study groups

| Parameter | Inadequate Iron Stores | Adequate Iron Stores | <i>P</i> Value |
|-------------------------------------|------------------------|----------------------|----------------|
| Serum Ferritin (ng/mL) | 120.1 \pm 115.8 | 182.4 \pm 121.1 | 0.09 |
| Transferrin Saturation (%) | 19.4 \pm 11.8 | 27.4 \pm 19.4 | 0.08 |
| Mean Cell Volume (fl) | 84.4 \pm 3.4 | 86.2 \pm 3.1 | 0.38 |
| Mean Cell Hemoglobin Content (g/dL) | 34.2 \pm 1.4 | 34.8 \pm 1.4 | 0.85 |
| Red Cell Distribution Width (%) | 12.9 \pm 1.2 | 12.7 \pm 1.3 | 0.77 |

deficiency was 7387 ± 300 , compared with 6094 ± 300 in patients with adequate iron (*P* = NS).

The mean values at baseline of the parameters studied as predictors of iron status are displayed in Table 3. There was no statistically significant difference between any of the parameters in the two study groups. The only tests with marginal significance were those for serum ferritin and transferrin saturation. The serum ferritin level was 120.1 ± 115.8 ng/mL in patients with iron deficiency, compared with 182.4 ± 121.1 ng/mL in patients with adequate iron (*P* = 0.09). The transferrin saturation was $19.4 \pm 11.7\%$ in patients with iron deficiency, and $27.4 \pm 19.4\%$ in patients with adequate iron (*P* = 0.08).

ROC were constructed for each of the parameters to evaluate the tradeoff between sensitivity and specificity as the screening cutoff value for the parameter changed. Table 4 demonstrates values used in the calculations for two of the parameters, serum ferritin and transferrin saturation. Figure 1 displays the superimposed ROC for the six tests. None of the parameters had a diagnostic cutoff value that offered optimal utility (combination of sensitivity and specificity both greater than 80%). The transferrin saturation at a value of <21% and a serum ferritin level of <150 ng/mL had the greatest utility of the tests studied. Various combinations of the parameters were studied to see if diagnostic accuracy could be strengthened. No combination was found to offer a significant improvement in utility.

DISCUSSION

The treatment of anemia in patients on hemodialysis is frequently hindered by the presence of inadequate iron (1). Negative iron balance develops in these patients because of decreased gastrointestinal absorption of iron (11), blood retention in the dialyzer and tubing, blood drawing for laboratory testing, and gastrointestinal bleeding (12). Assessment of iron status on an ongoing basis is an important component for the success of rHuEPO therapy (1). Unfortunately, the lack of clear standards for what constitutes abnormal iron tests in hemodialysis patients has been problematic. The goal of the study presented here was to determine the utility of commonly used diagnostic tests for determining the adequacy of available iron in these patients.

The utility of any diagnostic test can only be defined in terms of a second test, or other specific criterion chosen as a "gold standard." In the diagnosis of iron deficiency, bone marrow biopsy specimens stained for iron have traditionally served this function (2). We

TABLE 4. Sensitivity and specificity at various screening cutoff values for two of the iron indices tested (serum ferritin and transferrin saturation)

| Test | Sensitivity (%) | Specificity (%) |
|-------------------------------|-----------------|-----------------|
| Transferrin Saturation | | |
| <15% | 16 | 88 |
| <18% | 58 | 75 |
| <21% | 81 | 63 |
| <24% | 88 | 44 |
| <27% | 92 | 22 |
| <30% | 96 | 11 |
| Serum Ferritin (ng/mL) | | |
| <50 | 37 | 75 |
| <100 | 48 | 75 |
| <150 | 71 | 69 |
| <200 | 77 | 37 |
| <300 | 90 | 18 |
| <500 | 100 | 0 |

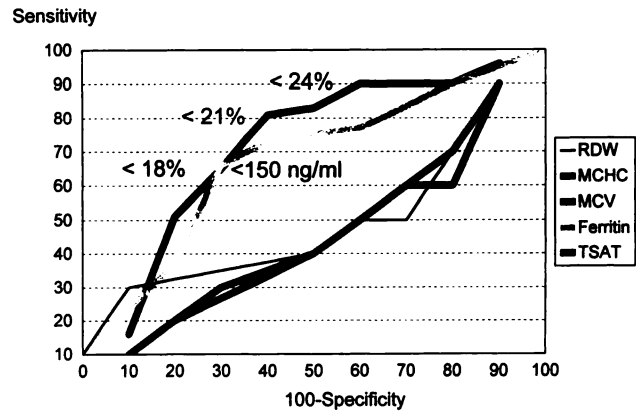


Figure 1. Receiver operator curve demonstrating the tradeoff between sensitivity and specificity as the screening cutoff values change for each of the iron indices. A test possessing high utility would have a value in the upper left-hand corner of the graph.

believe that this approach has hampered efforts to define useful clinical standards for iron indices in hemodialysis patients for several reasons: (1) the bone marrow biopsy cannot identify functional iron deficiency, the form of iron deficiency most common in hemodialysis patients. In this state, iron is present in bone marrow, but when erythropoiesis is stimulated to supraphysiologic levels by rHuEPO, iron immediately available to the bone marrow is insufficient, and iron deficient erythropoiesis ensues; (2) In this population, the test lacks specificity because of previous blood transfusions and administrations of intravenous iron dextran (2); and (3) it is subject to sampling error. The nephrologist in clinical practice tests iron status for one primary reason, to improve the therapy of the ESRD patient's anemia. The question the nephrologist needs answered can be stated as: Does the patient have adequate iron to optimize erythropoiesis, or is additional iron supplementation required? The ability of diagnostic tests to answer this question requires the use of a functional approach to define their utility. *Williams Hematology* states, "In the final analysis, the response to iron therapy is the proof of correctness of the diagnosis of iron deficiency anemia" (2). Administering a surplus of iron to patients, and observing their erythropoietic response, defines whether pretreatment iron was adequate. Patients in need of additional iron respond with an improvement in erythropoiesis rapidly after administration of intravenous iron (13,14). We have used this functional approach in a prior study in which only patients with a high pretest probability of iron deficiency were studied (10). To accurately define the sensitivity and specificity of a diagnostic test, unselected populations must be studied. Therefore, in the study presented here, we have used this functional approach to define the utility of iron indices in the general hemodialysis population.

Depending on how a test is used, certain character-

istics of its performance define its utility (15). The accuracy of a test is defined by its reliability and validity. Reliability refers to the reproducibility of test results, validity is defined by the sensitivity and specificity for a test. The cost of a test is determined both by the direct cost of application and indirect secondary costs attributable to actions taken in response to test results. The simplicity and safety of a test are self-defined characteristics (15). Testing iron status in hemodialysis patients serves the purpose of both screening and diagnosis, because unselected populations of patients are tested and the results are used to guide therapy. Therefore, an ideal test for this purpose should possess all of the attributes listed (accurate, inexpensive, simple, and safe). All of the tests we studied were relatively inexpensive, simple, and safe. None, however, was highly accurate. Two tests, the serum ferritin at a level of 150 ng/mL and transferrin saturation at a value 21%, had marginal utility. The serum ferritin test had sensitivity and specificity of only 71% and 69%, respectively. The transferrin saturation had a reasonable sensitivity of 81%, but a specificity of only 63%. Changing the screening cutoff value for either test resulted in an unacceptable loss of sensitivity or specificity. Because of the tests' marginal utility, we believe that iron indices in hemodialysis patients should be interpreted only in the context of the patient's responsiveness to rHuEPO therapy. In the patient who is rHuEPO responsive (achieves a target hematocrit value at a low rHuEPO dose), the diagnosis of inadequate iron is less critical. In these patients, we would suggest that the specificity of the iron indices used is more important than sensitivity. Therefore, in these patients, a transferrin saturation value of less than 18% or a serum ferritin level of less than 100 ng/mL should be the signal for intensifying iron management (specificity of >75% for inadequate iron stores). In contrast, in the patient who is resistant to rHuEPO therapy, the sensitivity of iron indices becomes more important. In these patients, either a transferrin saturation value of <27% or a serum ferritin level of <300 ng/mL yields greater than 90% sensitivity.

Several tests of iron status—such as serum iron, total iron binding capacity, red cell ferritin levels, and the percentage of hypochromic red blood cells—were not studied in this analysis. One test we have studied previously is that for zinc protoporphyrin. In a study of patients with high probability of iron depletion, we found this test to have good utility (10). The test has not gained clinical acceptance because of conflicting study results (16) and the expense of equipment required to perform the assay.

In conclusion, we have found that most common tests of iron status in hemodialysis patients lack

precision. Two tests, the serum ferritin at a level of <150 ng/mL and a transferrin saturation value of <21%, had marginal utility. We suggest that iron indices should only be interpreted in the context of the patient's responsiveness to rHuEPO therapy. In rHuEPO-responsive patients, we recommend that a serum of ferritin level of < 100 ng/mL or a transferrin saturation value of <18% be used as indicators of inadequate iron stores. Among rHuEPO-resistant patients, a transferrin saturation value of <27% or a serum ferritin level of <300 ng/mL should be used to guide management.

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