Assessing Iron Status: Beyond Serum Ferritin and Transferrin Saturation

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The increasing prevalence of multiple comorbidities among anemic patients with chronic kidney disease has made the use of serum ferritin and transferrin saturation more challenging in diagnosing iron deficiency. Because serum ferritin is an acute-phase reactant and because the inflammatory state may inhibit the mobilization of iron from reticuloendothelial stores, the scenario of patients with serum ferritin >800 ng/ml, suggesting iron overload, and transferrin saturation <20%, suggesting iron deficiency, has become more common. This article revisits the basis for the Kidney Disease Outcomes Quality Initiative recommendations regarding the use of serum ferritin and transferrin saturation in guiding iron therapy, then explores some of the newer alternative markers for iron status that may be useful when serum ferritin and transferrin saturation are insufficient. These newer tests include reticulocyte hemoglobin content, percentage of hypochromic red cells, and soluble transferrin receptor, all of which have shown some promise in limited studies. Finally, the role of hepcidin, a hepatic polypeptide, in the pathophysiology of iron mobilization is reviewed briefly.

Kidney Disease Outcomes Quality Initiative Guidelines

After considerable review of the literature, Kidney Disease Outcomes Quality Initiative (K/DOQI) anemia work groups in 1997, 2001, and 2006 decided that the serum ferritin and the transferrin saturation (TSAT) should be the primary tools for assessing iron management in patients with anemia and chronic kidney disease (CKD), including ESRD (1). The serum ferritin reflects storage iron, and absolute iron deficiency, according to the K/DOQI guidelines, correlates with serum ferritin <100 ng/ml. Absolute iron deficiency, the iron deficiency that is characterized by low or absent bone marrow staining for iron, is to be distinguished from functional or relative iron deficiency, which is defined as a response to intravenous iron with an increase in hemoglobin (Hb) or a decrease in erythropoiesis-stimulating agent (ESA) requirement. This can occur in patients with serum ferritin levels that are considerably higher than 100 ng/ml (1,2).

Iron overload, according to the K/DOQI guidelines, may occur in patients who have serum ferritins in excess of 800 ng/ml, but this is extremely variable. The literature that predates the use of ESA for the treatment of renal anemia, when patients were multiply transfused to maintain Hb levels that were compatible with a reasonable functional status, shows that many of those patients had serum ferritins in the 1000- to 2000-ng/ml range. On autopsy, there was very little evidence of tissue iron overload despite these high serum ferritin levels. The serum ferritin level of 800 ng/ml, which the K/DOQI guidelines propose as an upper limit for intravenous iron ther-

apy, is an opinion-based cutoff; it is not evidence based. The K/DOQI anemia workgroups chose this value to provide a considerable amount of buffer between the serum ferritin levels that we are used to dealing with in most of our dialysis patients and the serum ferritin levels of >2000 ng/ml in patients with hemochromatosis, in whom clinically relevant tissue iron deposition starts to occur (1).

The TSAT is the serum iron divided by the total iron-binding capacity (TIBC), which corresponds to circulating iron. The TIBC reflects transferrin, the protein to which virtually all iron in the blood is bound. The K/DOQI workgroups have determined that absolute iron deficiency, the absence or near absence of stainable iron in the bone marrow, correlates with TSAT <20% and that there is a risk for iron overload when the TSAT exceeds 50%. These also are opinion-based and not evidence-based guidelines.

Forms of Iron Deficiency

There is a spectrum of iron deficiency that occurs in patients with renal anemia, especially when they are treated with ESAs, because these agents stimulate the bone marrow to a supraphysiologic rate of red blood cell (RBC) production. The normal rate of iron delivery to the bone marrow, which is constrained by the amount of circulating iron, sometimes is insufficient to meet the iron demands of the ESA-stimulated marrow. Absolute iron deficiency, defined as TSAT <20% or serum ferritin <100 ng/ml, often will occur in patients who are on hemodialysis because of increased blood loss, from blood left in the dialyzer circuit, frequent blood sampling, low-grade gastrointestinal bleeding, multiple vascular access surgeries, *etc.* This also may be compounded by decreased oral iron absorption because of dietary restrictions, loss of taste for iron-rich foods, and hepcidin (see the Hepcidin section).

The condition that is known as functional iron deficiency or

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relative iron deficiency is unique to the population of patients who are being treated with these ESAs because their supra-physiologic rate of RBC production outstrips the ability of transferrin-bound circulating iron to provide adequate sub-strate for Hb synthesis. In these patients, the TSAT may be <20% as the hungry bone marrow strips iron off the circulating transferrin faster than the transferrin can replenish it with iron released from stores. The serum ferritin, which reflects iron stores, may be normal or elevated. This is a problem of supply and demand, not total body iron deficiency. The patient has amounts of iron in the body that may be normal for an indi-vidual who is not anemic and not on an ESA, but in the setting of ESA-driven bone marrow stimulation, the rate at which iron is released from stores and the rate at which iron is being delivered by transferrin to the erythroid marrow are insuffi-cient to keep up with RBC production. This may occur even when storage iron seems normal or elevated. This often precip-itates a clinical dilemma: Is the patient iron deficient or not?

An extreme case of functional iron deficiency is known as reticuloendothelial (RE) blockade and usually occurs in the setting of acute or chronic inflammation/infection. This often correlates with a high C-reactive protein level and/or a high erythrocyte sedimentation rate. Because of the inflammatory state and likely mediated by hepcidin (see below), the iron that is in RE storage gets "locked up" there and is not released to transferrin. As a result, transferrin-bound iron, which is re-flected by the TSAT, is low despite a normal or elevated ferritin. This condition is compounded by the fact that ferritin is itself an acute-phase reactant, like C-reactive protein, that is elevated in the setting of inflammation. This leads to the dilemma: Is the elevated serum ferritin level due to high iron stores, or is it due to the inflammatory state and therefore not a marker of storage iron? If the patient has low TSAT, as many do in this setting, then should he or she be treated with additional intravenous iron or not?

Limitations of Serum Ferritin/TSAT

That serum ferritin is an acute-phase reactant and that there are gender differences (normally lower in women) make fer-ritin somewhat less than an ideal test for determining iron deficiency. TSAT also has some acute-phase reactivity insofar as transferrin may be elevated in the setting of inflammation, which would lower the TSAT if circulating iron is constant. Transferrin may be low because of decreased transferrin syn-thesis in the setting of malnutrition and chronic disease, which would raise TSAT if circulating iron is constant. There also are

significant (17 to 70%) diurnal fluctuations in TSAT that make it difficult to interpret its value if the time of day at which the test is obtained varies from test to test (1,3).

The situation in which the TSAT is low and the serum ferritin is high often is encountered among hemodialysis patients. The serum ferritin may be elevated in this setting because of func-tional iron deficiency or RE blockade. The therapeutic dilemma is to decide whether additional iron supplements are indicated to bring the TSAT up into the K/DOQI target range, especially in patients who are not achieving the target Hb in the setting of ESA treatment. This is a risk *versus* benefit analysis: What is the risk to the patient's safety by giving him or her additional intravenous iron *versus* the benefit of providing additional iron for RBC production so that the patient can enjoy the physio-logic and quality of life rewards of a higher Hb level?

Ultimately, the diagnosis of iron deficiency should not be made in a clinical vacuum. It needs to be made by examining the entire patient, looking for the presence of inflammation or infection, especially if a dialysis catheter is present. Every cath-eter has a biofilm that can provoke an inflammatory response even in the absence of fever, leukocytosis, or positive blood cultures. In most of the patients with the dilemma of elevated serum ferritin and low TSAT, removal of the catheter should be considered a high priority. That issue notwithstanding, the question boils down to whether the patient needs more iron to support higher levels of Hb. An increase in the ESA dosage may seem to be the path of least resistance, because ESAs are perceived as more benign than intravenous iron. However, most of the patients with high serum ferritin and low TSAT levels already are demonstrating evidence of ESA resistance, so they already are on very high dosages of an ESA and still below the Hb target. That is the situation in which the clinician must come to terms with whether the patient should receive more iron and whether the serum ferritin and TSAT are providing the information that is needed to make that decision.

The accuracy of serum ferritin and TSAT is determined by their sensitivity and specificity. Sensitivity is the probability that a positive test will accurately identify iron status as defi-cient. Specificity is the probability that a negative test will accurately identify iron status as not deficient. The sensitivity and the specificity of these tests, as determined by three authors using different criteria, are summarized in Table 1 (4–6). The conclusion is that a TSAT of 20% seems to be relatively good in terms of sensitivity, meaning that few patients are truly iron deficient with a TSAT much higher than 20%, but a ferritin

Table 1. Sensitivity and specificity of iron measures[a]

Study	Gold Standard	Serum Ferritin/TSAT Cutoff	Sensitivity Serum Ferritin/TSAT	Specificity Serum Ferritin/TSAT
Fishbane *et al.* (4)	Functional	<100 ng/ml/<21%	48%/81%	75%/63%
Tessitore *et al.* (5)	Functional	<100 ng/ml/<19%	35%/59%	78%/78%
Kalantar-Zadeh *et al.* (6)	BM	<200 ng/ml/<20%	41%/88%	100%/63%

[a]The utility of a diagnostic test can be defined only in terms of a second test, a "gold standard." TSAT, transferrin saturation; Functional, response to iron therapy; BM, bone marrow biopsy with iron staining.

cutoff of 100 or even 200 ng/ml tends to miss close to a majority of patients who ultimately may respond to intravenous iron.

Reticulocyte Hb Content

The reticulocyte Hb content (CHr) is a measure of the amount of Hb in the reticulocytes, which are the RBC that are just 1 or 2 d old. Accordingly, the amount of Hb in the reticulocytes is a reasonably good reflection of how much iron was available to the bone marrow for incorporation into new red blood cells a few days before. Rather than examining the Hb content of the entire RBC population that may be anywhere between 1 and 120 d old, the CHr provides a snapshot of how much iron was available for RBC production in a clinically relevant timeframe. This theoretically should be a reasonably good marker, based on the premise of whether iron was available for RBC production. The CHr is widely available on many of the same multichannel hematology analyzers that do the complete blood count and is a separately billable test that costs $4.25.

Two studies that examined the sensitivity and the specificity of CHr in diagnosing iron deficiency are summarized in Table 2 (7,8). The CHr compared favorably with serum ferritin and TSAT in predicting a response to intravenous iron in both studies. Fishbane *et al.* (9) examined 157 patients and compared ferritin/TSAT with CHr as a trigger for treatment with intravenous iron dextran, 100 mg × 10 consecutive hemodialysis treatments, for a total of 1 g. In group 1, the investigators treated the patients with serum ferritin levels <100 ng/ml and TSAT <20%. In group 2, the investigators used CHr <29 pg and then explored a subset with a higher cutoff of 30 pg. They found that the CHr was less variable than the TSAT or the ferritin and that it was more accurate. In a subsequent study, Fishbane *et al.* (9) determined that a CHr cutoff of 29 pg tended to miss a number of patients who ultimately responded to intravenous iron. The authors concluded that a cutoff of 32 pg showed a much greater utility, and a majority of the patients who had iron therapy on the basis of a CHr <32 pg had an average of 23% reduction in their erythropoietin requirements.

Percentage of Hypochromic Red Cells

Another alternative iron marker is percentage of hypochromic red blood cells (PHRC), which is a test of the concentration of Hb in RBC, as opposed to the Hb content as in CHr. The CHr is the absolute amount in picograms of Hb in each reticulocyte.

Because PHRC is based on the Hb concentration in RBC, it takes into account the absolute amount of Hb as well as the size of the cell. The big problem with the utility of this test in the United States is that the blood samples cannot be shipped because RBC tend to expand while they are stored. Because most of the large dialysis chains in the United States use national laboratories and because there is considerable storage time between the time when the blood samples are collected and the time when these analyses eventually are done, which may be as long as 18 to 24 h, this has not turned out to be a particularly useful test in the United States. Conversely, in Europe, where most of the laboratories are local and where the storage time is not as much as it is in the United States, PHRC has turned out to be a relatively useful test.

Table 3 shows the results of two studies that compared PHRC with CHr in the diagnosis of iron deficiency, using different cutoffs and different standards (5,10). Cullen's data (10) demonstrated the most commonly used PHRC cutoff of 10% to be inferior to a CHr cutoff of 26 pg. Not surprising, the very low CHr cutoff had 100% specificity (no false positives) yet still a reasonably good sensitivity. Neither of these investigators used the Fishbane-recommended (9) CHr cutoff of 32 pg, which one would predict would increase sensitivitiy and decrease specificity *versus* the cutoffs used. The conclusion is that PHRC probably is comparable to CHr in terms of its utility, but the storage issue will remain a limiting factor with regard to its widespread use in the United States.

Soluble Transferrin Receptor

The soluble or serum transferrin receptor test (sTfR) is based on the fact that erythroblasts in the bone marrow will increase the presentation of membrane transferrin receptor in the setting of iron deficiency. If a patient is not receiving sufficient iron and erythropoiesis is being stimulated by an ESA, then increased transferrin receptors will become expressed on the erythroblasts, some of which come off and will be detectable in the circulation. The sTfR correlates with this membrane expression of the transferrin receptor and also tends to be elevated in the presence of increased erythroid activity. The treatment of a patient with an ESA, by increasing the total erythroblast mass, also will increase the sTfR. When a patient is noted to have an elevated sTfR, the clinician must determine whether it is due to iron deficiency or because the patient is on an ESA or has

Table 2. CHr in the assessment of iron status[a]

Study	Standard	Other Comparisons	Sensitivity	Specificity
Mittman *et al.* (7)	Functional (1% ↑ in corrected reticulocyte index after intravenous iron 500 mg)	Serum ferritin < 100 ng/ml TSAT < 20%	CHr 78% serum ferritin 38% TSAT 50%	CHr 71% serum ferritin 53% TSAT 60%
Chuang *et al.* (8)	Functional (30% ↓ in EPO dose after intravenous iron 2200 mg over 24 wk)	Serum ferritin < 300 ng/ml	CHr 78% serum ferritin 83%	CHr 87% serum ferritin 57%

[a]CHr, reticulocyte hemoglobin (Hb) content; EPO, erythropoietin.

Table 3. PHRC verus CHr in the assessment of iron status[a]

Study	Standard	Comparisons	Sensitivity	Specificity
Cullen *et al.* (10)	TSAT <15%	PHRC > 10% CHr < 26 pg	PHRC 64% CHr 73%	PHRC 77% CHr 100%
Tessitore *et al.* (5)	↑ Hb >1.9 g/dl in response to intravenous iron (992 mg over 8 wk)	PHRC > 6% CHr ≤ 29 pg	PHRC 78.1% CHr 52.9%	PHRC 78.1% CHr 52.9%

[a]PHRC, percentage of hypochromic red blood cells.

increased erythroblastic activity, and that alone is increasing the membrane and ultimately the serum expression of the transferrin receptor.

The results are somewhat mixed in using sTfR in patients with ESRD to diagnose iron deficiency. It does seem to be a reasonable index of erythropoietic activity (11,12). If one is trying to determine whether an ESA is having its intended effect of stimulating bone marrow RBC production, before an increase in reticulocytes is noted and well before the Hb rises, then an increase in the sTfR may be the first detectable measure. It is not affected by inflammation (13), which one would think would make sTfR a more reliable test than serum ferritin, but Fernandez-Rodriguez *et al.* (14) found sTfR to be less accurate than serum ferritin. Those authors demonstrated an sTfR sensitivity of 70% and a specificity of 59% at a cutoff of 2.6 mg/L. Tessitore *et al.* (5) demonstrated an sTfR sensitivity of 81% and a specificity of 71% at a cutoff of 1.5 mg/L. Nonetheless, there is little broad consensus regarding the use of sTfR in the diagnosis of iron deficiency until larger studies are done. The test is not widely available, which also limits its utility.

Hepcidin

Hepcidin is a peptide that is produced by the liver for iron homeostasis. It is a crucial mediator for iron absorption and mobilization. If storage iron is elevated, then the liver synthesizes hepcidin, which feeds back to the gastrointestinal tract and to the placenta in pregnant women, preventing additional exogenous iron absorption. Hepcidin also inhibits the release of iron from the RE system to circulating transferrin. Under normal physiologic circumstances, when iron stores are replete, it is important to protect the organism from iron overload by preventing the entry of additional iron into the body and maintaining the appropriate balance of storage and circulating iron. Hemochromatosis results from a genetic defect in hepcidin activity such that the individual lacks the normal feedback suppression of intestinal iron absorption and iron overload results. Hepcidin activity in normal individuals is increased in the setting of inflammation/infection, primarily through the release of IL-6 by Kupffer cells in the liver (15). This explains the phenomenon of RE blockade in which storage iron is not released to circulating transferrin, resulting in a high serum ferritin and low TSAT level. Not surprising, there is a significant correlation between hepcidin and serum ferritin because both are acute-phase reactants (16).

Conclusion

TSAT and serum ferritin have remained the favored markers for assessment of iron status through three iterations of the K/DOQI anemia guidelines because of their widespread availability, extensive literature base, and familiarity. The frequent paradox of high serum ferritin and low TSAT has made it desirable to seek alternative iron markers to predict better whether subsets of patients will respond to iron therapy. CHr is accurate and reproducible, but the optimal cutoff value still is not final. On the basis of Fishbane's data, somewhere in the neighborhood of 32 pg seems reasonable. The test is available in the national laboratories that serve the large dialysis chains and many independent dialysis facilities in the United States. It may not be available on the hematology analyzers that serve hospital-based facilities and other regional laboratories. The storage and shipping issues for laboratory specimens make PHRC problematic for widespread use in the United States, despite data that put its accuracy on a par with CHr. The literature on sTfR is limited, and there is no consensus regarding cutoff. It is not widely available, but a more robust evidence basis may increase its application in the future. Further study is needed to determine the role of hepcidin in assessing iron pathophysiology and determining the presence or absence of an RE blockade situation. For the foreseeable future, the alternative tests will prove most useful not as screens or primary diagnostic markers for iron deficiency but to evaluate better the challenging cases.

Ultimately, the decision of whether to use intravenous iron therapy in a particular patient should be made in the context of that patient's overall condition. If the benefits outweigh the risks, as in a patient who has failed to achieve the target Hb despite large dosages of ESA and in whom the TSAT is low and there is no evidence of active infection, then a trial of intravenous iron might be warranted despite a high serum ferritin level. Despite many concerns regarding the safety of intravenous iron since the first iteration of the K/DOQI anemia guidelines 9 yr ago, there have been few data in the literature from which to conclude that patient outcomes have been adversely affected by the use of intravenous iron within the original K/DOQI parameters. The more data at a clinician's disposal with which to guide therapy, the better, which is why alternative iron markers are welcome and will continue to be explored.

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