Serum Ferritin in Chronic Kidney Disease: Reconsidering the Upper Limit for Iron Treatment

Steven Fishbane,* Kamyar Kalantar-Zadeh,† and Allen R. Nissenson†
*Department of Medicine, Winthrop-University Hospital, Mineola, New York; and †Division of Nephrology, Department of Medicine, David Geffen School of Medicine, UCLA, Los Angeles, California

ABSTRACT

Intravenous iron treatment in hemodialysis patients improves the response to recombinant human erythropoietin (rHuEPO) and facilitates achievement of targets for hemoglobin and hematocrit. Excessive treatment, however, could expose patients to risks related to iron overload and oxidative stress. Therefore international treatment guidelines generally recommend that intravenous iron be discontinued when serum ferritin is greater than 500–1000 ng/ml. In this article we explore the relevant issues that inform the decisions as to what levels of serum ferritin are used as the upper limit for treatment. We conclude that the current published literature is inadequate for developing evidence-based guidelines. Clinical judgment is critical to properly weigh the risks and benefits of intravenous iron treatment in the context of the individual patient.

Intravenous iron treatment is an important component of anemia therapy for patients with chronic kidney disease (CKD). Since excessive treatment could lead to iron overload, with potentially harmful consequences, there is a need to monitor therapy with blood tests that reflect the body's iron load. Both the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI) Anemia Treatment Guidelines and the European Best Practice Guidelines for the Management of Anaemia in Patients with Chronic Renal Failure recommend that iron treatment be withheld if the serum ferritin concentration rises to more than 800 ng/ml or transferrin saturation is greater than 50% (1,2).

Recently there has been increased discussion and controversy as to what the upper limit for these tests in relation to iron treatment should actually be. In part, interest in the targets has been fueled by the finding of rapidly increasing measured iron stores in American hemodialysis patients. In 1993 the Dialysis Mortality and Morbidity Wave 1 study reported a mean serum ferritin in 2613 hemodialysis patients of 154 ng/ml (3). After publication of the K/DOQI guidelines in 1997, there was greater recognition of the need for improved iron management in these patients. The result was a sharp increase in the use of intravenous iron. As a result, by 2001, the U.S. Renal Data System (USRDS) found that mean serum ferritin had increased to 526 ng/ml (4). It is unclear whether the 242% increase in serum ferritin has either benefited patients with higher hemoglobin levels or more efficient use of recombinant human erythropoietin (rHuEPO), nor is there specific evidence for patient harm.

Address correspondence to: Steven Fishbane, MD, 200 Old Country Rd., Suite 135, Mineola, NY 11501, or e-mail: sfishbane@winthrop.org.

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The purpose of this article is to review our current knowledge as to the upper boundaries of serum ferritin with respect to iron safety and efficacy. We will not consider transferrin saturation, since potential iron overload from intravenous iron treatment impacts most directly on iron storage, measured more directly by serum ferritin (an indicator of storage iron) than by transferrin saturation (a measure of circulating iron). In addition, we will not review newer iron tests, such as percentage hypochromic red blood cells or reticulocyte hemoglobin content, since iron overload in relation to these parameters has not been extensively studied.

Biology of Serum Ferritin

Terrestrial life is highly dependent on the use of iron and dioxygen to drive energy storage processes. The combination of iron and oxygen, however, is a corrosive process that, under physiologic conditions, yields harmful compounds such as rust and oxygen free radicals (5). Ferritin is nature's solution to the difficult chemistry of iron and oxygen, storing iron in a safe, soluble manner that allows for regulated release of iron as needed for normal metabolism. These processes are critical for life; deletion of the gene for ferritin leads to early embryonic death (6).

Ferritin is one of the body's two major iron storage compounds (hemosiderin is the other). It is a sphere with a diameter of 13 nm and has a center cavity that is connected to the surface of the molecule by six channels. The outer shell is composed of 24 heavy (H) and light (L) polypeptide chains folded into four-helix bundles, and the cavity can hold as many as 4500 Fe(III) atoms. When fully loaded with iron, the molecule has a molecular weight of approximately 800,000 kDa (7). The transport and processing of iron within the ferritin complex

are incompletely understood. It is known, however, that the molecule has 8 sites for entry of iron, 3–24 sites for the oxidation of iron to its Fe(III) state, sites for translocation and mineral attachment and mineralization, and 8 sites for iron exit from the molecule (5).

Under normal conditions of body iron loading, most somatic cells contain little ferritin. In contrast, cells responsible for specialized iron functions, such as storage, may contain large amounts of ferritin. In these cells a large number of inactive ferritin messenger RNA (mRNA) resides in the cytoplasm. When iron enters the cell the mRNA is processed and ferritin polypeptide subunits are rapidly produced (8). The process is regulated by the interaction between noncoding sections of the mRNA (iron responsive elements) and cytoplasmic iron regulatory proteins (9).

The processes by which ferritin enters the circulation are not completely known. Serum ferritin differs from tissue ferritin, containing only negligible amounts of the H-polypeptide subunit. In addition, serum protein is more highly glycosylated and has, in the past, been considered to be devoid of iron (10). Recently, however, it has become possible to measure the iron content of serum ferritin, and it is clear that serum ferritin does in fact contain considerable quantities of iron (11). It has been demonstrated that the iron content of serum ferritin varies in relation to iron status, inflammation, and tissue injury (11,12). Whether the iron content of serum ferritin has potential diagnostic implications remains to be determined.

During episodes of inflammation, serum ferritin concentrations become elevated as a manifestation of the acute-phase response (13). Increased cellular release of ferritin into the circulation does not appear to be due to increased synthesis, as mRNA for the H and L subunits of apoferritin are not increased (14). Rather, during inflammation, increased translation of preformed mRNA may be the key process. Interleukin (IL)-1 induces ferritin mRNA translation via a translational enhancer region located upstream from the segments coding for the polypeptide chains (14).

The increase in serum ferritin during inflammation hinders the ability to diagnose iron deficiency. In rheumatoid arthritis, a disease of chronic inflammation, iron deficiency is present in more than half of patients, yet serum ferritin levels are normal or increased (15). In patients with CKD, serum ferritin levels are almost always elevated compared to nonuremic subjects, and are frequently elevated further as a result of inflammation (16–18). This fact may help to explain the finding that serum ferritin has poor sensitivity for the diagnosis of iron deficiency in patients with CKD (19–22). In fact, when serum ferritin is used as per the K/DOQI guidelines (1) (values less than 100 ng/ml for the diagnosis of iron deficiency), the sensitivity is only approximately 50% (in contrast, the test's specificity is fairly good). Restated, reliance on a serum ferritin level of 100 ng/ml for iron treatment decisions will leave at least half of iron-deficient patients untreated.

Similar considerations hinder the ability of serum ferritin to diagnose iron overload in patients with CKD. High levels of serum ferritin may indicate iron overload; however, in many patients results may be elevated due to inflammation even if iron stores are not increased. In one recent study, Kirchbaum (23) found that approximately 34% of hemodialysis patients with elevated serum ferritin had inflammation as the probable cause of the increased level. Another recent study found significantly higher serum ferritin values in malnourished and/or inflamed hemodialysis patients, and showed that in those hemodialysis patients whose serum ferritin was greater than 800 ng/ml, serum C-reactive protein (CRP) was significantly higher than in those with a serum ferritin value less than 800 ng/ml (24).

Choosing an Upper Limit of Serum Ferritin for Iron Treatment

As discussed above, the K/DOQI anemia guidelines recommend a ceiling serum ferritin level of 800 ng/ml for treatment with intravenous iron, with the guideline being designated as opinion based (1). There are various factors that are important in selecting an "upper limit" for serum ferritin (from this point on we will use the simplified term "upper limit" to represent the level of serum ferritin at which intravenous iron treatment should be withheld). In order to simplify the analysis, we will approach the relevant factors via consideration of the question: Is the current level of 800 ng/ml appropriate or should the upper limit be reduced to 500 ng/ml? Such a discrete approach will help simplify and focus the discussion. In addition, the two possible levels are relevant because one represents the current K/DOQI guideline (800 ng/ml), and the other has been discussed as a possible replacement level (500 ng/ml) and is a level that has been associated in early observational studies with increased infection risk (19,25–27).

Issues related to iron safety are particularly important when considering an upper limit to serum ferritin. The greatest safety concern with the use of intravenous iron has in the past been the risk for anaphylaxis (28,29). The higher the upper limit, the greater the amount of intravenous iron treatment and the accompanying risk for anaphylaxis. However, with the nondextran forms of intravenous iron, iron sucrose and iron gluconate, the risk for anaphylaxis is greatly reduced (30–32). Other iron safety issues, however, are still relevant, including the risks of iron overload, infection, tissue oxidation, and atherosclerosis.

Iron overload is a state in which iron accumulates in the body over time, challenging the capabilities of tissue storage systems and potentially resulting in damage to tissues and organs. Ali et al. (33) performed an autopsy study of 50 hemodialysis patients in the pre-EPO era. They found a surprisingly large number of subjects had severe iron tissue overload (36%) and found that serum ferritin was not a very good predictor of the degree of iron overload. Of importance is that despite the excess iron in tissues, there was no evidence of tissue damage or pathology. Recently Canavese et al. (34) studied 40 hemodialysis patients, most of whom had recently had intravenous iron discontinued due to high serum ferritin. They used a new technology, superconductive quantum

interference device magnetic resonance imaging (SQUID-MRI), which noninvasively tests for tissue iron overload. The test has been found to correlate well with measuring iron load from tissue samples obtained by liver biopsy. Canavese et al. (34) found that 37.5% of patients had severe iron overload, similar to the proportion found by Ali et al. (36%). Of interest is that neither serum ferritin nor treatment with intravenous iron or dosage of intravenous iron correlated well with a risk for iron overload.

Taken together, Canavese et al. and Ali et al.'s work demonstrate that iron is often present in excess in the liver tissue of patients on hemodialysis. The explanation for this excess of hepatic iron is unclear. It is intuitive that intravenous iron treatment would contribute to tissue iron loading, but as noted, Canavese et al. did not find a relationship between iron treatment and dose and tissue iron burden. Alternatively, hepatic iron storage may be increased due to inflammation and/or reticuloendothelial iron blockade. In these states the body shifts iron pools, with increased iron in storage and less in circulation. Therefore it is possible that the increased hepatic iron stores in hemodialysis patients are not pathologic and do not harm tissues. Indeed, it is exceedingly uncommon to find hemodialysis patients with liver damage due to iron overload, and Ali et al. (33) found little iron pathology despite severe iron overload at autopsy.

The effects of iron overload are best understood in the disease state hemosiderosis and the related genetic disorder hemochromatosis. In these diseases, many years of excessive iron storage lead gradually to tissue and organ damage. The heart, joints, pancreas, liver, and other organs may be severely affected (35). The level of serum ferritin at which organ damage occurs has recently been studied. Morrison et al. (36) performed live biopsies in 182 patients with hemochromatosis. The mean serum ferritin among patients with histologic evidence of cirrhosis was 4411 ng/ml and no patient with serum ferritin less than 1000 ng/ml had evidence of liver pathology. Indeed, organ damage in hemochromatosis is very rare when serum ferritin is less than 1000 ng/ml and patient age is less than 40 years (36–40). In patients with CKD, there is a far shorter duration of exposure to high serum ferritin levels. Given this fact, and the absence of organ damage at ferritin levels less than 1000 ng/ml, it is very unlikely that the current K/DOQI upper limit for serum ferritin of 800 ng/ml represents any potential risk of iron overload-induced tissue damage.

The risk of infection related to higher serum ferritin levels or due to intravenous iron treatment itself extends from the observation that iron is essential for the growth of microorganisms (41–43). Rogers et al. (42) and Bullen et al. (43) found that injection of bacteria into animals did not cause infection unless iron was injected first, in which case overwhelming sepsis developed. Furthermore, intravenous iron treatment has been associated with reduced white blood cell function (44–46). These and other findings establish that it is at least plausible that iron treatment or higher serum ferritin levels could promote infection risk.

Several published studies have evaluated the relationship between serum ferritin and infection risk in hemodialysis patients. Generally serum ferritin levels greater than 500 ng/ml have been found to be associated with an increased risk of infection (25–27); these studies are from the pre-rHuEPO era. No relationship between infection and either intravenous iron doses or serum ferritin levels has been found since widespread use of rHuEPO has minimized red blood cell transfusions. In addition, it is unclear whether the relationship indicates causality—that is, greater body iron stores causing increased infection risk. As discussed above, serum ferritin is a potent acute-phase reactant, and infection itself or clinical conditions that increase the risk of infection may increase serum ferritin levels independent of iron status. Even with the most sophisticated multivariate analyses, it may be impossible to clearly elucidate this issue. Of note is that the only available prospective, multicenter study found no association between serum ferritin or cumulative iron dose and the risk of bacteremia among hemodialysis patients (47). Given the conflicting study results and the limitations in study design, it is difficult to use the current published data to rigorously inform decisions as to the upper limit for serum ferritin.

Another iron safety issue that is relevant to considerations of the serum ferritin upper limit is that of potential oxidative tissue damage related to increased iron storage or to intravenous iron treatment itself. Iron is a powerful oxidizing substance, and the body has highly conserved mechanisms to protect tissues from iron exposure and subsequent oxidative damage. Ferritin itself, as discussed above, sequesters iron in a deep central core in the molecule. In circulation, iron is safeguarded by transferrin, and release of iron to tissues is a highly regulated receptor-mediated process (48,49). Excessive iron storage could result in an overburdening of tissue-based ferritin and hemosiderin, and as a result, free iron could accumulate in tissues and organs, resulting in oxidative damage. Indeed, in hemochromatosis this may be the mechanism of tissue injury.

However, as discussed above, it is unlikely that iron storage would be oversaturated to this extent in patients with CKD. Rather, oxidative injury is more likely to occur as a direct result of intravenous iron treatment itself. After intravenous iron injection, ideal processing would have the drug removed from the circulation by the reticuloendothelial system (RES) before any iron is released into plasma. The drug should be processed in the RES, with subsequent regulated release of iron into the circulation for carriage by transferrin. Any direct and immediate release of iron after injection into the circulation could potentially overwhelm the ability of serum transferrin to bind the iron. Free, non-transferrin-bound iron may then be present in circulation, with the potential to cause oxidative tissue damage and to induce the production of reactive oxygen species.

Initial studies of intravenous iron and the release of non-transferrin-bound iron may have used faulty methodology, with drug-bound iron being included in the measurement of serum free iron (50). More recent studies have used more precise analytic techniques to measure free iron. Parkkinen et al. (51) found that injection of 100 mg of iron sucrose caused transferrin saturation to rise sharply within 10 minutes, indicating the immediate release of iron from the drug into the circulation. Subjects

with lower levels of serum transferrin were significantly more likely to have free iron present (51). Similarly Kooistra et al. (52) and Rooyakkers et al. (53) found that intravenous injection of iron sucrose led to abundant free iron in circulation in hemodialysis patients and normal volunteers, respectively. In the latter study, free iron release was associated with increased reactive oxygen species in plasma and reduced flow-mediated forearm blood flow (53). Moreover, Roob et al. (54) found that iron sucrose injections in hemodialysis patients led to free iron in serum and evidence of oxidation, which could be reduced by pretreatment with vitamin E. Recent studies have added evidence for an association between intravenous iron sucrose treatment and protein oxidation and a possible association with accelerated atherosclerosis (55,56). In another recent report, oxidized fibrinogen was detected in plasma after intravenous ferric gluconate injection (57).

Taken together, the body of evidence indicates that intravenous iron injection does appear to be associated with some free, non-transferrin-bound iron appearance in plasma, with a resulting increase in oxidative stress. The impact on patients' health and clinically relevant outcomes is not yet known. As for infection risk, it is not yet possible to fully quantify the risk of iron-induced oxidative tissue injury or to relate the risk to specific levels of serum ferritin.

A possible association between iron storage and accelerated cardiovascular disease risk has been proposed based on iron's oxidative properties and the relationship between oxidation and atherosclerosis risk (58). The theory was first proposed by Sullivan (59), with the hypothesis that iron deficiency might protect against atherosclerotic disease. Given the high prevalence of cardiac disease among patients with CKD (60) and the frequent use of iron supplementation in this population, any relationship between the two may be clinically important. However, there are few reports addressing this subject specifically in patients with CKD, so we must look to studies performed in other populations, albeit a potentially misleading approach. Salonen et al. (61) studied the risk for myocardial infarction in 1931 middle-aged Finnish men. Serum ferritin levels greater than 200 ng/ml were found to independently predict the risk for cardiac disease. In contrast, Magnusson et al. (62) studied more than 2000 subjects and found no association between serum ferritin and cardiac risk. Similarly, other results from the literature have been mixed, but with more negative than positive studies (62–71). A natural model to explore the relationship exists in the form of the disease hemochromatosis. Miller and Hutchins (71) studied patients with this disease and found a rate of severe coronary artery disease of only 12%, compared to 39% of controls with normal iron stores. Taken together, these studies would seem to indicate that, in the range of serum ferritin under consideration as the upper limit for iron treatment (500–800 ng/ml), cardiovascular risk is probably not an important factor.

Another relevant issue in this discussion may be the possibility that reducing the serum ferritin cutoff for intravenous iron treatment in CKD from 800 ng/ml to 500 ng/ml might lead to undertreatment of iron-deficient patients. Indeed, given the poor sensitivity of serum ferritin, it is plausible that at a serum ferritin of 500 ng/ml, some patients may still be iron deficient. There have been several published studies that have explored the diagnostic accuracy of serum ferritin at these levels. Our group studied 50 hemodialysis patients based on response to intravenous iron and found that the test's sensitivity was 90% at 300 ng/ml and 100% at 500 ng/ml (19). Similar results were found by Fernandez-Rodriguez et al. (72), who studied 63 hemodialysis patients. The sensitivity of serum ferritin was 92% at 300 ng/ml and 98% at 600 ng/ml (72). Tessitore et al. (21), in a study of 125 hemodialysis patients, found serum ferritin to be highly sensitive at values greater than 300 ng/ml. Chuang et al. (73) studied 65 hemodialysis patients and found that only 17% of iron-deficient subjects had serum ferritin greater than 300 ng/ml.

Taken together, these studies would seem to indicate that iron deficiency is probably quite rare when serum ferritin is greater than 500 ng/ml. However, such a conclusion is probably flawed. All such studies require the presence of a "gold standard" to measure the test—serum ferritin—against. In these studies, two such "gold standards" were used: 1) bone marrow biopsy samples stained for iron, and 2) functional hemoglobin or reticulocyte response to a course of intravenous iron. Sensitivity was defined by the ability of serum ferritin to predict iron deficiency as defined by these references.

There are problems with both approaches. Since much of the iron deficiency in CKD is functional in nature, bone marrow testing probably grossly underestimates the need for intravenous iron treatment. In contrast, the functional approach provides useful information about the response to and need for intravenous iron treatment. However, all studies used relatively short-term follow-up and short courses of iron treatment. Therefore these studies tell little about the intermediate and long-term need for iron. That this is an important consideration is supported by a study reported by Besarab et al. (74). Although not specifically designed to evaluate the performance of serum ferritin, the results yield important information. Patients were treated with weekly doses of intravenous iron for more than a year. The greatest response to rHuEPO was found in patients with mean serum ferritin of approximately 600 ng/ml, with many having considerably higher levels (74). We would conclude therefore that the current literature provides inadequate information to fully inform decisions as to the possible efficacy of intravenous iron treatment when serum ferritin is greater than 500 ng/ml.

Conclusion

Intravenous iron therapy is a key component of the care of patients with CKD, particularly those on hemodialysis. Without it, rHuEPO treatment may often be suboptimal, resulting in serum hemoglobin below target levels, with an associated increased risk of adverse outcomes (1). The decision as to the upper limit for serum ferritin at which iron treatment should be withheld requires a careful balancing between potential benefits

and risks to patients. As discussed, current knowledge with respect to iron safety is incomplete and inadequate. The literature relating to iron deficiency and the efficacy of intravenous iron treatment at higher levels of serum ferritin is similarly inadequate for guiding evidence-based practice. Therefore we conclude that 1) it is not possible to have an evidence-based guideline for the upper limit of serum ferritin; 2) no specific level of serum ferritin, not 500 ng/ml or 800 mg/ml, or some other number, should be stated as an upper limit for iron treatment. Given the lack of support from the literature, any attempt to set an upper limit would be arbitrary and would not serve to improve the quality of treatment. When making iron treatment decisions when serum ferritin is greater than 500 ng/ml, however, we would encourage the clinician to balance the possibility of benefit against considerations of risk in the context of the individual patient. The clinician must carefully weigh factors such as the current hemoglobin level and rHuEPO dose, the patient's current clinical status, and the results of other iron testing such as transferrin saturation.

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