Should We Give IV Iron to HD Patients with Infection?

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Hemodialysis patients experience heavy losses of iron due to a combination of recurrent blood loss in dialysis circuit and routine laboratory tests and impaired intestinal iron absorption occasioned by elevated hepcidin level. For this reason, in compliance with prevailing guidelines, hemodialysis patients are routinely treated with IV iron products. Recent studies have demonstrated the lack of validity of the iron biomarker limits stablished by clinical guidelines for the use of IV iron in ESRD patients. This has led to excessive use of IV iron products and epidemic of iron overload in ESRD populations in most countries except Japan which has a far more conservative guideline for the use of IV iron. Plasma contains only a tiny amount (3-4 mg) of iron bound to transferrin which serves as a safe vehicle for transport of iron in the body. IV iron products are administered as bolus injections of 100 to 1000 mg which overwhelm the transferrin pool and dramaticaly raise plasma non-transferrin bound and catalytically active iron triggering activation of oxidative and inflammatory cascades.

Infectious complications are common and a major cause of morbidity and mortality in ESRD patients maintained on dialysis modalities. Iron overload can increase the risk and severity of infections by simultaneously enhancing bacterial growth and virulence and suppressing host's defense against microbial invasion. Under normal conditions the microorganisms' access to the body's iron is limited by encasment of iron in binding proteins in extracellular (Transferrin and lactoferrin) and intracellular (hemoglobin, myoglobin, enzymes, etc.) compartments. However, by providing ample supply of iron, the dramatic rise in non-transferrin-bound iron following IV iron injection can enhance microbial growth and virulence. In addition to promoting bacterial growth and virulence excess iron compromises host defense against microbial invasion by impairing neutrophil and T-cell functions. Together, these events increase the risk and severity of bacterial infections. In addition to its harmful effects on bacterial infections, iron overload intensifies the severity and adverse outcomes of viral hepatitis. Hepatitis C and B infections are common in hemodialysis patients. Iron has been shown to significantly amplify replication and virulence of hepatitis C virus and elevated liver tissue iron has been shown to intensify severity of hepatitis C infection and favour development of cirrhosis and the risk of hepatic cancer in the general population. Similarly, increased liver tissue iron adversely affects the course of hepatitis B infection. IV iron administration in compliance with the accepted guidelines in hemodialysis patients has been shown to increased transaminase levels after the third month of therapy indicating amplification of hepatocellular injury by IV iron therapy.

Given the demonstrated effects of excess iron in promoting bacterial and viral growth and virulence, suppressing host defense capacity, and intensifying bacterial and viral infections in general population and ESRD patients, caution should be exercised in the use of IV iron in infected ESRD population.

In 12 HD patients with central vein catheters, the administration of IV iron sucrose was followed by the release of NTBI and more frequent signs of bacterial growth in half of them (especially in those with TSAT >30%) [43-Barton Pai A Pai J Depczynski CR, Mercier, RC. Non- transferrin-bound iron is associated with enhanced Staphylococcus aureus growth in hemodialysis patients receiving intravenous iron sucrose. Am J Nephrol 2006; 26: 304–309]. Teehan *et al.* [44- Teehan GS Bahdouch D Ruthazer Ret al Iron storage indices: novel predictors of bacteriemia in hemodialysis patients initiating intravenous iron therapy. Clin Infect Dis 2004; 38: 1090–1094] found that HD patients receiving IV iron despite replenished iron indices are at increased risk for bacteremia. In a retrospective cohort study of HD patients, Brookhart *et al.* [45-Brookhart MA Freburger JK Ellis ARet al Infection risk with bolus versus maintenance iron supplementation in hemodialysis patients. J Am Soc Nephrol 2013; 24: 1151–1158] compared the safety of iron bolus dosing (100 mg in at least two consecutive treatments) to maintenance dosing (low-dose

administration every 1–2 weeks to maintain iron stores). Patients receiving the bolus were at higher risk of infection than those on a maintenance dose, especially if they had a central vein catheter or had had a recent infection. Similarly, the DOPPS study showed a trend towards an increased infection-related mortality in prevalent HD patients treated with > 300 mg of IV iron [14-Bailie GR Larkina M Goodkin DAet al Data from the Dialysis Outcomes and Practice Patterns Study validate an association between high intravenous iron doses and mortality. Kidney Int 2015; 87: 162–168]. A meta-analysis of 24 clinical trials also found an increased risk of infection with IV iron compared with oral or no iron treatment [Litton E Xiao J Ho KM. Safety and efficacy of intravenous iron therapy in reducing requirement for allogeneic blood transfusion: systematic review and meta-analysis of randomised clinical trials. BMJ 2013; 347: f4822]. Conversely, a prospective observational study of 985 patients failed to demonstrate a relationship between infection and serum ferritin or IV iron dosing [Hoen B Paul-Dauphin A Kessler M. Intravenous iron administration does not significantly increase the risk of bacteriemia in chronic hamodialysis patients. Clin Nephrol 2002; 57: 457–461]. Of note, the frequency and the amount of iron administered were significantly higher in those who developed bacteraemia than in those who did not.

Altogether, the evidence relating IV iron with increased infection risk is scarce, mainly because of the heterogeneity of the studies and bias in treatment indications. Nevertheless, we agree with the KDIGO guidelines [Kidney Disease: Improving Global Outcomes Anemia Work Group. KDIGO clinical practice guideline for anemia in chronic kidney disease. Kidney Int Suppl 2012; 2: 279–335], which suggest not administering IV iron during active systemic infections.

Recently, a meta-analysis of 2658 patients from 24 single-arm studies and 10 randomized clinical trials did not demonstrate an increased risk of adverse events including infections, cardiac events

and mortality [14- Bailie GR Larkina M Goodkin DAet al Data from the Dialysis Outcomes and Practice Patterns Study validate an association between high intravenous iron doses and mortality. Kidney Int 2015; 87: 162–168]. Of note, these data were obtained from an exploratory analysis restricted to only two randomized clinical trials (359 analysable patients). The median duration of IV iron administration was 16 weeks, ranging from 2 to 96 weeks. However, even when the sample size of clinical trials is put together in a meta-analysis, it remains largely insufficient to test hard end points.

Iron is an essential nutrient for microbial organisms. To acquire iron microorganisms produce siderophores which are small molecules with high affinity for iron to chelate and internalize iron. Microorganisms produce a variety of siderophores which are encoded by different genes. It should be noted that siderophores play a major role in the microbial virulence and formation of biofilms by different microbes. A recently introduced phosphate binding compounds commonly used to limit hyperphosphatemia in CKD patients is ferric citrate complex which is prescribed at doses containing 2-4 g of iron per day. Given the critical role of iron in microbial growth and virulence, the present study was undertaken to test the hypothesis that the heavy load of iron delivered to the colon with administration of ferric citrate in CKD may result in significant changes in the gut microbiome. To this end fecal microbiome was determined in rats with CKD treated with or without ferric citrate for 6 weeks. They observed more cases of acute cardiocerebrovascular disease (HR: 6.02) and hospitalization (HR: 2.77) in the high-dose IV iron group, and increased risk of infections in both the low (HR: 1.78) and high (HR: 5.22) IV iron-treated groups[13. Kuragano T, Matsumura O, Matsuda A et al. Association between hemoglobin variability, serum ferritin levels, and adverse events/mortality in maintenance hemodialysis patients. Kidney

International, 2014; 86(4): 845-854]. High ferritin levels (consistently above 100 µg/L, in accordance with Japanese guidelines [13]) were associated with the risk of acute cardiocerebrovascular disease (HR: 2.22), infections (HR: 1.76) and death (HR: 2.28) [13. Kuragano T, Matsumura O, Matsuda A et al. Association between hemoglobin variability, serum ferritin levels, and adverse events/mortality in maintenance hemodialysis patients. Kidney International, 2014; 86(4): 845-854]. The result of the Japanese study is in keeping with a recent US study showing that iron maintenance therapy at 200 mg/month is not associated with an increased short-term risk of infections, contrasting that encountered with bolus monthly doses of 700 mg [81. Brookhart MA, Freburger JK, Ellis AR et al. Infection risk with bolus versus maintenance iron supplementation in hemodialysis patients. JASN 2013; 24(7): 1151-1158].These latter results are in line with the findings of a recent controlled trial of IV iron-sucrose versus oral iron in non-dialyzed CKD patients, showing increased serious cardiovascular and infectious events in IV iron-treated patients as compared to those receiving oral iron [82. Agarwal R, Kusek J, Pappas M. A randomized trial of intravenous and oral iron in chronic kidney disease. Kidney Int advance online publication, 17 June 2015; doi:10.1038/ki.2015.163].

Iron overload ~~may~~ might affect several lineages of immune cells, leading to an increased risk of infection, as shown in some epidemiological studies: these effects could include CD4+ T cell depletion associated with shortened cell lifespan, CD8+CD28- T lymphocyte expansion, impaired phagocytic activity, and microbial killing of polymorphonuclear leukocytes and monocytes [92. Pahl MV, Vaziri ND. Immune function in chonic Kidney disease. Chapter 4 pp 285-297. In Chronic Renal Disease, Kimmel P and Rosenberg M Editors Academic Press-Elsevier, 2015]. In addition, since iron is an essential element for bacterial multiplication and virulence, iron overload due to high doses of IV iron may increase the risk and severity of infections.

One of the micronutrients that the vast majority of bacterial species require for their growth and metabolism is iron (Andrews, et al., 2003). However in the human body, iron availability to microbes is generally extremely limited, due to innate iron withholding mechanisms that aim to prevent growth of pathogenic invaders (Cassat & Skaar, 2013). Such a tightly regulated system of nutritional immunity is not known for the lumen of human gut.

However, also in the gut lumen with high amounts of dietary iron present, gut microbes have to deal with the stress of iron limitation as the presence of freely available "unbound" iron is probably limited due to the environmental conditions of the colon lumen. The balance of bound and unbound iron can be (re)disturbed by the oral administration of supplementary iron, a common strategy to treat iron deficiency, which is known to cause alterations of the gut microbiota composition and metabolism (see section 7&8). In Figure 1 we summarised the (iron-related) metabolic stress factors that will be discussed throughout this review.

Effects of iron availability on the small intestinal microbiota are likely to be different from the effects on the colonic microbiota. It is important to realize that the small intestinal microbiota has a much lower density of residing microbes, and that its composition and metabolism appears to fluctuate quickly in response to dietary input (Zoetendal, et al., 2012). Iron may play a large role within this environment where also pathogenic bacteria like Salmonella spp, Vibrio cholera and pathogenic E. coli may colonise. However, relatively little is known about the small intestinal microbiota, as it is much less accessible compared to the colonic (faecal) microbiota, which makes it difficult to study (Zoetendal, et al., 2012).

bacteria have evolved multiple iron uptake mechanisms to be able to fulfil their average need of 10-7 to 10-5 M for optimal growth. Several mechanisms of iron uptake were previously described for various bacterial species, and were most extensively studied in E.

coli. In the latter bacterium, iron can be taken up in both its ferrous and ferric form. One of the mechanisms of ferrous iron uptake is via the Feo-uptake system (Andrews, et al., 2003). Moreover, ferric iron (which is insoluble) can be reduced to ferrous iron via an extracellular reductase, after which it can be taken up in the ferrous form (Cowart, 2002). Alternatively, ferric iron can be taken up as ferric citrate, or bound to excreted bacterial siderophores, small iron chelators with a very high affinity for iron (Andrews, et al., 2003, Miethke & Marahiel, 2007). After excretion, bacteria re-internalise the ferric-siderophore-complex via binding to specific receptors and transporters. For example the ferric-enterobactin-complex binds to the outer membrane receptor FepA of gram-negative bacteria, after which it is transported to the periplasm, a process dependent on the TonB system that provides the energy for this transport. Next, the ferric-enterobactin-complex is further transported into the cytoplasm by ABC-transporters (Chu, et al., 2010). Direct uptake of haem-bound iron or scavenging of haem with haemophores are other specialized ways of iron acquisition (Andrews, et al., 2003). It is important to mention that haem-iron is much more bioavailable for humans compared to non-haem iron (Zimmermann & Hurrell, 2007), that haem causes oxidative and cytotoxic stress in the gut lumen and that haem can alter the (mouse) gut microbiota composition (IJssennagger, et al., 2012, IJssennagger, et al., 2013).

Iron & bacterial virulence

It has been known for a long time that the scarcity of iron generally limits bacterial growth whereas iron availability often enhances bacterial replication and the expression of virulence factors in pathogenic bacteria, thereby exaggerating infection (Payne & Finkelstein, 1978, Weinberg, 1978, Litwin & Calderwood, 1993, Bullen, et al., 2005). Furthermore, knockout

studies have shown that iron uptake mechanisms such as FeoB are required for full virulence in vivo (Tsolis, et al., 1996, Janakiraman & Slauch, 2000, Boyer, et al., 2002, Naikare, et al., 2006). Bacteria sense the availability of iron in the environment and a change in the amount of available iron (for example, from high to low) during invasion of the host is an important signal for the expression of virulence genes. On the one hand, under low iron conditions, the production of siderophores is derepressed, and also the production of toxins may be upregulated (Litwin & Calderwood, 1993, Payne, 1993). On the other hand, in vitro studies have shown that freely available iron can directly induce the expression of virulence genes in S. Typhimurium; especially bacterial adhesion to intestinal epithelial cells seems to be stimulated when iron is highly available (Bjarnason, et al., 2003, Kortman, et al., 2012). Thus both high and low iron conditions seem to be able to stimulate different aspects of bacterial virulence.

To acquire iron in the iron limiting conditions created by the host, in particular during an inflammatory response (hepcidin) invading) pathogens may express receptors for host transferrin and lactoferrin, but more well-known is the secretion of iron scavenging siderophores (Andrews, et al., 2003, Cairo, et al., 2006). These molecules mostly have a very high specificity and affinity for ferric iron and can therefore compete with iron bound to host proteins. To illustrate, transferrin has an affinity constant for ferric iron of about 1022, while the association constant for the siderophore enterobactin for ferric iron is approximately 1051 (Carrano & Raymond, 1979, Aisen & Listowsky, 1980). We note that (slightly) different association constants are circulating in literature for transferrin and enterobactin. Fortunately, the fight for iron does not end here as the mammalian host

has evolved a mechanism to sequester some bacterial ferric-siderophore-complexes with the innate defence peptide lipocalin-2 (neutrophil gelatinase-associated lipocalin (NGAL), siderocalin, 24p3, uterocalin). By binding to the ferric-enterobactin-complex, lipocalin-2 prevents the uptake of ferric enterobactin by bacteria, thereby enhancing nutritional immunity (Flo, et al., 2004). However, certain pathogenic bacteria can evade this sophisticated host defence mechanism by the production of stealth siderophores' (Allred, et al., 2013). For instance, S. Typhimurium and pathogenic E. coli can produce a C-glucosylated form of enterobactin, termed salmochelin, which escapes from the binding by lipocalin-2 (Hantke, et al., 2003, Raffatellu & Baumler, 2010). Another example is the production of aerobactin, which does not bind to lipocalin-2 either, and is produced by for instance Klebsiella, Shigella, some Salmonella serovars and pathogenic E. coli strains (Wooldridge & Williams, 1993, Flo, et al., 2004).

Iron speciation and bacterial iron uptake mechanisms in the colon

As far as we know, bacteria synthesize siderophores only when the availability of iron in their surround environment is limited. In the colonic lumen, large amounts of iron are regularly present, mostly constituted by excess dietary iron not absorbed in the duodenum This is illustrated by the high concentration of iron found in feces of British adults on a standard western diet and in weaning infants fed with complementary solid foods: approximately 100 µg Fe/g wet weight faeces, which is roughly equal to 1.8 mM, and which is much more than the minimal iron requirement of most bacterial species (Pizarro, et al., 1987, Lund, et al., 1999). Moreover, the tight mechanisms of iron withholding in systemic sites do not known to play a role in the gut lumen. Nevertheless, iron speciation and the

potential presence of lactoferrin, lipocalin-2 and other yet unidentified defence proteins in the colon mucosa probably contribute to the limitation of iron at this site. Notably, lipocalin-2 is only expressed at a low level under healthy conditions (Cowland & Borregaard, 1997) and would be more effective when iron in the lumen is limited and thus when siderophores are produced. Due to the nature of foods and of the oxygenic and acidic environment of the stomach, most of the dietary non-haem iron that enters the intestine is in the ferric form (Jacobs & Miles, 1969). When the pH in the duodenum and small intestine rises, the solubility of ferric iron decreases (Figure 3). Simultaneously, the increase in pH favours the oxidation of ferrous iron in the presence of oxygen. Further downstream, the reduced environment in the colon may favour the reduction of iron into the ferrous form (Hedrich, et al., 2011). Also the colonic microbiota may influence the valence state of iron by the action of extracellular reductases (Cowart, 2002, Takeuchi, et al., 2005) (Figure 3). The possible effect of colon luminal pH on iron solubility was discussed in a study from Romanowski et al., in which surgically injured mice with the oral provision of a phosphate buffer at pH 7.5 showed an increased colonic pH compared to mice provided with the same buffer at pH 6.0. The expression of bacterial siderophore systems was upregulated within the neutral pH-group, possibly due to a decreased solubility of iron in the intestinal lumen at a higher pH (Romanowski, et al., 2011). It should however be noted that phosphate also influences siderophore production and that iron is prone to form complexes with phosphate, which could have contributed to their findings. These in vivo results are nevertheless consistent with an in vitro study, which showed that lowering of the pH increased iron uptake (Salovaara, et al., 2003).

Iron speciation and solubility in the colon is extremely difficult to predict as many factors can influence this process and the various iron species in the lumen are not easy to measure accurately.

Consequently, the amount iron in the colon lumen that is readily available to bacteria is also difficult to estimate. Besides oxygen and pH, which are two main influencers of iron speciation, different food components can affect the valence state and solubility. The most famous food component in this matter is **ascorbic acid (vitamin C)**, which chelates iron and reduces ferric iron to its ferrous form (Figure 3). For this reason, ascorbic acid is also known for its positive effect on iron absorption by the host (Miret, et al., 2003). Other organic acids such as **citrate** are known to form a soluble chelate with iron and may hereby prevent precipitation of ferric iron when the pH rises after passage through the stomach (van Dokkum, 1992) (Figure 3). Nonetheless, the neutral to mildly acidic pH of the intestinal lumen favours precipitation with hydroxides and complex formation with mucins, certain amino acids, proteins, and other food components. Therefore, iron species such as iron oxides, iron hydroxides, iron phosphates and iron carbonates may be found in the intestinal lumen, but we do not know how accessible these insoluble forms of iron are for bacteria (Simpson, et al., 1992, Cremonesi, et al., 2002) (Figure 3). Bacteria may solubilise some of these forms by lowering the external pH, by reducing the iron to the more soluble ferrous form, or by binding to siderophores (Ratledge & Dover, 2000, Andrews, et al., 2003). Ligands that potentially bind to iron and play a role in the colonic lumen are summarised in Supplementary Table 2. In Supplementary information 1 we envisage how orally administered iron will end up in the colon after ingestion. In conclusion, both ferrous and ferric forms of iron are likely to be present in the colon and there are reasons to believe that iron availability to the colonic microbiota is normally limited.

5.2 Quest for food-bound iron sources (gut microbiota paper to continue)

5.2 Quest for food-bound iron sources

Because iron speciation in the colon is difficult to predict and it is likely highly variable, not much is known about the accessibility of colonic luminal iron. Nonetheless, here we discuss a few iron sources that are normally not easy to access, but may become available to certain members of the gut

Unlike conventional oral iron preparations, ferric citrate has recently been shown to be effective in increasing serum ferritin, hemoglobin, and transferrin saturation values while significantly reducing IV iron and ESA requirements in patients treated with HD. Ferric pyrophosphate citrate is a novel iron salt delivered by dialysate; by directly reaching transferrin, its obviates the need for storing administered iron and increases transferrin saturation without increasing serum ferritin levels. Ferric pyrophosphate citrate trials have demonstrated effective iron delivery and stable hemoglobin levels with significant reductions in ESA and IV iron requirements. To date, the long-term safety of using these routes of iron administration in patients receiving HD has not been compared to IV iron and therefore awaits future investigations.

Infection risk

Iron overload is an increasingly recognized clinical situation among hemodialysis patients. The liver is the main iron storage site in humans, and the liver iron concentration correlates closely with total body iron stores in patients with secondary hemosiderosis and genetic hemochromatosis. Hepatic magnetic resonance imaging (MRI) is now considered the gold standard method for estimating and monitoring iron stores in secondary hemosiderosis and genetic hemochromatosis. Recent studies using quantitative MRI to estimate liver iron stores have suggested a strong link between the IV iron dose and the risk of iron overload in dialysis patients, and have challenged the reliability of currently accepted iron biomarker cutoff values and clinical guidelines, especially regarding recommended iron doses (4, 5).

. recently published reviews have tended to favor the possibility of an association and even **advocated the withholding of intravenous iron in the setting of active infection (4–6).** Kidney Disease Outcomes Quality Initiative (KDOQI) addressed the topic of iron and infection risk in their 2000 anemia guidelines and concluded that maintaining a serum ferritin within the recommended range was unlikely to pose a risk for bacterial infection in patients with chronic kidney disease (CKD) (19). However, subsequent international CKD anemia guidelines advised caution with using intravenous iron in the setting of infection (16,17), and some recommended avoiding or withholding intravenous iron in patients with systemic infection (18,20).

The most recent of these guidelines was published by Kidney Disease Improving Global Outcomes (KDIGO) in 2012 and advised avoiding intravenous iron in patients with active systemic infections (18-Kidney Disease Improving Global Outcomes (KDIGO) Anemia Work Group. KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease. Kidney international Supplement. 2012; 2:279–335-). However, this recommendation was not graded and was based on the biologic plausibility that iron may increase the risk of infection (21. Wessling-Resnick M. Iron homeostasis and the inflammatory response. Annu Rev Nutr. 2010; 30:105–122. [PubMed: 20420524]; 22. Appelberg R. Macrophage nutriprive antimicrobial mechanisms. J Leukoc Biol. 2006; 79(6):1117– 1128. [PubMed: 16603587]; 23. Byrd TF, Horwitz MA. Interferon gamma-activated human monocytes downregulate transferrin receptors and inhibit the intracellular multiplication of Legionella pneumophila by limiting the availability of iron. J Clin Invest. 1989; 83(5):1457–1465. [PubMed: 2496141]; 24. Mencacci A, Cenci E, Boelaert JR, Bucci P, Mosci P, Fe d'Ostiani C, Bistoni F, Romani L. Iron overload alters innate and T helper cell responses to Candida albicans in mice. J Infect Dis. 1997; 175(6):1467–1476. [PubMed: 9180188]; 25- Nairz M, Theurl I, Ludwiczek S, Theurl M, Mair SM, Fritsche G, Weiss G. The co-ordinated regulation of iron homeostasis in murine macrophages limits the availability of iron for intracellular Salmonella typhimurium. Cell Microbiol. 2007; 9(9):2126–2140. [PubMed: 17466014]) as well as limited data from observational studies in hemodialysis patients (26. Hoen B, Paul-Dauphin A, Hestin D, Kessler M. EPIBACDIAL: a multicenter prospective study of risk factors for bacteremia in chronic hemodialysis patients. J Am Soc Nephrol. 1998; 9(5):869– 876. [PubMed: 9596085] 27. Hoen B, Paul-Dauphin A, Kessler M. Intravenous iron administration does not significantly increase the risk of bacteremia in chronic hemodialysis patients. Clin Nephrol. 2002; 57(6):457– 461. [PubMed: 12078950] 28. Teehan GS, Bahdouch D, Ruthazer R, Balakrishnan VS, Snydman DR, Jaber BL. Iron storage indices: novel predictors of bacteremia in hemodialysis patients initiating intravenous iron therapy. Clin Infect Dis. 2004; 38(8):1090–1094. [PubMed: 15095212]). Despite the lack of a "clear answer" as to whether intravenous iron increases infection risk in CKD patients, the Work Group erred on the side of caution and considered iron administration to be harmful in the setting of infection. However, it did not discuss the possibility that withholding iron out of concern for infection may lead to iron deficiency, which may in itself pose a risk for infection (.29. Yetgin S, Altay C, Ciliv G, Laleli Y. Myeloperoxidase activity and bactericidal function of PMN in iron deficiency. Acta Haematol. 1979; 61(1):10–14. [PubMed: 217216] 30. Moore LL, Humbert JR. Neutrophil bactericidal dysfunction towards oxidant radical-sensitive microorganisms during experimental iron deficiency. Pediatr Res. 1984; 18(8):789–794. [PubMed: 6089085)

Reexamination of the safety of intravenous iron is especially timely in light of the recent **introduction of the bundled ESRD reimbursement system in 2011, which appears to have prompted increased utilization of intravenous iron (32).**

Iron and Infection: Biologic Plausibility

Iron participates in important oxidation-reduction reactions that are essential for life (33). "Free" (i.e., unbound) iron (Fe2+ and Fe3+) contributes to the formation of reactive oxygen species (34,35), which are important for phagocyte function (1,36). Iron is required for proper host defense against infection, and iron deficiency has been associated with impaired neutrophil function (1,29–31).

However, an **excess of iron has also been linked to impaired neutrophil and T cell function** and **promotion of microbial growth** in in vitro and in vivo studies involving animals and humans, although the duration of this effect has not been well established as the longest follow-up times were 2–3 days (37,38).

In **neutrophils** from healthy volunteers incubated with ferric compounds (39–41) and nondialysis patients with iron overload (42), impairments in polymorphonuclear (PMN) cell migration, phagocytosis, and survival have been observed. There is also evidence of impaired function in neutrophils obtained from dialysis patients with iron overload (43–45) or treated with intravenous iron (38,46). While much of the literature on the biologic basis for increased infection risk due to iron has focused on neutrophil function, there is also evidence of an influence of iron on **T cell function**. In mice that had been iron overloaded with intraperitoneal injections of iron dextran, failure to mount a Th1-mediated protective immune response to Candida albicans infection has been observed (24). Treatment with deferoxamine (iron chelator) restored the Th1 response and ability of iron overloaded mice to survive Candida albicans infection.

In addition to its effects on PMN and T cell function, iron may promote **bacterial growth** directly. Several in vitro and in vivo studies in animals and humans have shown iron to be a growth factor for numerous bacteria and other pathogens (47). For example, the risk of death in mice inoculated intraperitoneally with Pasturella septica increased by 9–10 fold when lysed red blood cells or purified hemoglobin were first mixed with the bacteria as a source of iron prior to injection (48). **Humans have developed mechanisms for withholding iron from microorganisms as part of their host defense against infection** (49). The iron-binding proteins **transferrin** (found in highest concentrations in plasma and lymphatic fluids) and **lactoferrin** (found predominantly in mucosal secretions and phagocytic granules) (49) are thought to sequester iron away from microorganisms and provide a form of nonspecific immunity against infection (33,36,49). However, some bacteria can still compete for iron by producing siderophores (iron chelators), while others can directly acquire iron from transferrin via a membrane-bound transferrin receptor (1).

Intravenous iron may result in over-saturation of host iron-binding proteins, **increasing free iron availability and promoting bacterial growth (37,50–52)**. In a study of twelve hemodialysis patients injected with 100 mg of iron saccharate, mean transferrin saturation and catalytically active iron concentration increased from baseline by 5 minutes, remained elevated for at least 210 minutes, and returned to baseline by 2–3 days (37). After inoculation with Staphylococcus epidermidis, bacterial growth at 9 to 24 hours was higher in serum obtained 210 minutes after iron saccharate injection compared to serum obtained before iron saccharate injection. The higher bacterial

growth found in the 210 minutes sample was abolished by addition of iron-free apotransferrin to the serum, indicating that Staphylococcus epidermidis growth was dependent on the presence of non-transferrin-bound serum iron

Commented [NV1]:

Fe overload and viral hepatitis. The prevalence of HCV infection among dialysis patients in Japan, Europe and North America during the 2012-2015 period was found to be 8.7% in the DOPPS study. **Increased hepatic tissue iron has been shown to exert a deleterious role in the course of hepatitis C, favour development of fibrosis and cirrhosis and possibly increase the risk of liver cancer in the general population. Regular loss of blood in the hemodialysis circuit, in routine blood sampling for laboratory tests (for uremia monitoring), and in gut due to uremic enteropathy, invariably results in iron deficiency for which patients are commonly treated with intravenous (IV) iron preparations. Data on the effects of IV iron in hemodialysis patients with hepatitis C are limited (2 studies) and strongly suggest that parenteral iron may contribute to hepatocellular injury.** The reduction of excess liver and body iron stores by phlebotomy has been shown to ameliorate the course of hepatitis C among patients who cannot receive interferon. In addition phlebotomy improve histologic lesions and potentiate the salutary effects of interferon. In fact phlebotomy was an accepted therapy for hepatitis C infection in the USA and Japan before the advent of antiviral agents (16, 17, 18). In a systematic review, Fanchini et al reported that patients treated with phlebotomy followed by interferon therapy (in 7 studies with 373 patients) had an odds ratio of 2.32 (95% CI: 0.96-6.24) for achieving a sustained virologic response as compared to patients receiving interferon alone (19). Similarly, in a meta-analysis of 6 randomized controlled trials including 367 patients, Desai and co-workers compared interferon therapy alone to interferon plus concomitant phlebotomy (20). The authors concluded that phlebotomy significantly improved the virologic response to interferon (odds ratio: 2.69: 95% CI: 1.60-4.52)(20). **Iron, at concentrations of 50 and 100 µmol of FeSO4, has been shown to enhance hepatitis C virus replication about ten-fold in cultured human hepatocytes (non-neoplastic PH5CH8 cells) within 48 hours. These changes were first observed 12 hours after supplementation and continued for 72 hours (**Kakizaki S[1], Takagi H, Horiguchi N, Toyoda M, Takayama H, Nagamine T, Mori M, Kato N. Iron enhances hepatitis C virus replication in cultured human hepatocytes. Liver. 2000 Apr;20(2):125-8**).** The opposite is found with very high iron levels. The Fe-induced increase in viral replication and virulence is amplified by iron mediated oxidative stress injury in the liver.

1) Data on the use of IV iron in hemodialysis patients with HCV infection are scarce. Two studies from Turkey (one with 89 HCV-infected patients, the other with 66 patients) showed that IV iron, given according to current guidelines, significantly increased transaminase levels (ALT and AST) after the third month of therapy, leading the authors to conclude that parenteral iron therapy might contribute to hepatocellular injury in these patients (Kahraman S, Yilmaz R, Genctoy G, Arici M, Altun B, Erdem Y, Yasavul U, Turgan C. Efficacy and safety of intravenous **iron** therapy for HCV-positive haemodialysis patients. Nephron Clin Pract. 2005;100(3):c78-85; Ozdemir A, Yalinbas B, Selamet U et al. Relationship between iron replacement and hepatic functions in hepatitis C virus-positive chronic haemodialysis patients. Nephrology (Carlton). 2005; 10(5): 433-437).

2) Elevated liver tissue iron (usually mild or moderate) is encountered in about 40 % of patients with chronic hepatitis B and has been linked to the severity of liver disease (higher activity scores and fibrosis) (Martinelli AL, Filho AB, Franco RF et al. Liver iron deposits in hepatitis B patients: association with severity of liver disease but not with hemochromatosis gene mutations. J Gastrenterol and Hepatol. 2004; 19(9): 1036-1041).

Ferritin and Infection in Hemodialysis Patients

Thirteen studies with sample sizes ranging from 61 to 2,662 have examined the link between serum ferritin and infection in hemodialysis patients (Table 1). **Nine of these studies** found that **high serum ferritin** (typically defined as **>500 or 1,000 ng/mL or, equivalently, µg/L**) was associated with higher incidence of bacterial infection or infection-related mortality (11,13,28,53–58). The incidence of bacterial infection ranged from 0.34 to 0.59 infections per patient-year (in studies evaluating the rate of infection) and 0.93% to 61.9% (in studies evaluating the proportion with infection) in the higher serum ferritin groups and 0.09 to 0.18 infections per patient-year and 0% to 37% in the lower serum ferritin groups (13,28,53– 56,58). In absolute terms, these studies suggest an excess of 16 to 50 infections per 100 patient-years in the higher compared with the lower serum ferritin groups. In studies that expressed the association between serum ferritin and bacterial infection as ratios, **higher serum ferritin was independently associated with a 1.5 to 3.1-fold higher incidence of bacterial infection or infection-related mortality (11,28,57,58).**

However, four studies did not observe an association between higher serum ferritin and infection. There was no significant difference in bacteremia-free tunneled catheter survival comparing patients with serum ferritin >500 and ≤500 ng/mL (59), the proportion with infection, pneumonia, or cellulitis/carbuncle comparing patients with serum ferritin >600 and <600 ng/mL (12), or a significant association between serum ferritin and infection-related mortality (60). In addition, a team of French investigators (Hoen et al.) found that serum ferritin was not a significant risk factor for bacteremia (26), a result that is in contrast to their prior finding that higher serum ferritin was an independent risk factor for bacterial infection (57). The authors attribute these discrepant results to the lower prevalence of iron overload (defined as a

serum ferritin >1,000 ng/mL) in the more recent study (5%) compared to the older study (over 10%).

It is also worth noting that the prevalence of erythropoietin use was much lower in the earlier (16.1%) than later (51.5%) Hoen et al. study. In hemodialysis patients with transfusional iron overload, erythropoietin treatment lowers serum ferritin and transferrin saturation and improves phagocytosis and killing of Yersinia enterocolitica (61). Thus, it is possible that the increased prevalence of erythropoietin use may have contributed to the lack of association between ferritin and bacterial infection observed in the later Hoen et al. study. Additionally, the earlier study (57) measured serum ferritin at the time of first bacterial infection (or at the end of the study period if no bacterial infection was present) whereas the later study (26) assessed the association of baseline ferritin with subsequent infection. Inferences from studies evaluating the association between serum ferritin and infection, such as the Hoen et al. studies, are confounded by the fact that ferritin is an acute phase reactant that may be elevated in the setting of infection (62). Thus, it is possible that the results from the earlier study were biased in favor of finding an association between serum ferritin and infection given the timing of ferritin measurement.

Aside from the limitations of serum ferritin as a predictor variable, studies involving ferritin also had limitations in the definition of their outcome variable. Some of these studies focused solely on the outcome of sepsis/bacteremia (26,28,54,56,59) or excluded certain types of bacterial infections (55) and may have underestimated the association between serum ferritin and bacterial infection. Many of these studies were descriptive and either did not control for confounding (12,13,53–55,59) or made very limited attempts to do so (56). Some of these studies were conducted before erythropoiesis-stimulating agents and intravenous iron were widely used and during a time when blood transfusions were the mainstay of treatment for anemia of ESRD (53–57). In this setting, it would be expected that iron overload was mainly due to repeated blood transfusion, which typically provides a larger iron load than intravenous iron administered according to international guidelines (6) and may have a different impact on immune function and infection risk than iron overload caused by intravenous iron (63,64). Thus, the relevance of these studies to the current practice environment, in which widespread erythropoiesis-stimulating agent use has reduced the need for repeated blood transfusions and

prevalence of iron overload (3,65), is unclear. Finally, all but four studies involved patients from countries other than the U.S. (11,13,26,53–57,59), limiting generalizability to U.S. hemodialysis patients. The percentage of studies finding an association between serum ferritin and infection risk was higher in studies conducted outside than inside of the U.S. (78% vs. 50%).

Iron Usage and Infection in Hemodialysis Patients

Twenty-four studies have evaluated the association between iron usage and infection in hemodialysis patients (Table 2). While not the primary aim of the analyses, two randomized controlled studies have addressed the association between any iron usage or more "aggressive" iron repletion and infection in hemodialysis patients.

In the Dialysis Patients'Response to IV Iron with Elevated Ferritin (**DRIVE**) study, anemic (hemoglobin ≤11 g/dL) hemodialysis patients with serum ferritin 500–1,200 ng/mL and transferrin saturation ≤25% were randomized to ferric gluconate (125 mg intravenously for eight consecutive dialysis sessions) or no iron (7). The number of infections over the **6-week** study was **not substantially different** between the two groups.

Another trial randomized hemodialysis patients with hemoglobin ≥9.5 g/dL, serum ferritin 150–600 ng/mL and transferrin saturation 19–30% to iron dextran to achieve and maintain a transferrin saturation of 30– 50% or to maintain a transferrin saturation of 20–30% over **a 6 month** period (66). There was one death due to infection in the former group in a patient with multiple risk factors for infection. Each group had one admission for an infectious etiology.

However, the majority of studies (**twenty-two**) evaluating the association between iron usage and infection in hemodialysis patients are **observational** and have yielded conflicting results. The sample sizes ranged from 21 to 309,219, and the iron exposure and infectious outcome variables were heterogeneously defined. **Twelve of these studies found an association between** any iron usage or higher dose or frequency of iron usage and infection or infection related mortality (9,11,15,59,60,67–73).

While a few studies evaluated the infection risk of any iron usage (11,60,72), most of these studies incorporated iron dose or frequency into their iron exposure variables. In two large

studies, higher frequency and higher dose of intravenous iron were independently associated with 14 to 35% higher risk of infection-related mortality (67,68).

In another large study, compared with a reference mean dose of 25–34 mg of intravenous iron per dialysis treatment, high dose (>34 mg per dialysis treatment) and no iron were independently associated with higher hazard of infection-related mortality whereas low dose (1–13 mg per dialysis treatment) was independently associated with lower hazard of infection-related mortality (70).

Other studies observed a significantly higher iron dosage (at study conclusion) in patients who had developed catheter-related bacteremia compared to those who had not (59) and a significantly higher mean number of intravenous iron vials per month among those with a higher number of hospitalizations for vascular access infections (71)

A relatively small study (n=111) observed a significantly higher rate of bacterial infection with higher iron sucrose dose but not higher frequency dosing (69).

In contrast, a much larger study (n=117,050) found a relatively modest but higher independent hazard of infection-related hospitalization or mortality (HR 1.08, 95% CI 1.05–1.11) and an excess of 26 of these events per 1,000 patient-years with bolus compared to maintenance intravenous iron (15). They also found a higher independent hazard of infection-related hospitalization or mortality (HR 1.05, 95% CI 1.02–1.08) and an excess of 13 of these events per 1,000 patient-years with high (>200 mg/month) compared with low (1–200 mg/month) dose intravenous iron. However, compared with no intravenous iron, receipt of maintenance (HR 0.99, 95% CI 0.97–1.02) and low dose (HR 0.99, 95% CI 0.97–1.02) intravenous iron were not significantly associated with infection-related hospitalization or mortality.

Only **a couple of studies have examined differences among formulations of iron**. In one study, there was a significantly higher rate of bacteremia with iron sucrose compared to ferric gluconate, although the association between iron dose and bacteremia was uncertain (73).

Another study observed a significant association between iron sucrose and catheter-related sepsis; iron dextran did not have a significant association with catheter-related sepsis (9).

The remaining ten studies did not find an association between iron and infection (8,10,12,14,26–28,58,74,75).

Iron (intravenous or oral) usage within the last 6 months was not significantly associated with hazard of bacteremia (26), and in a follow-up study by the same team of investigators analyzing a subset of the original cohort, intravenous iron and weekly intravenous iron dose were not significantly associated with hazard of bacteremia (27).

Two studies observed that 3-month cumulative intravenous iron dose was not significantly associated with incidence of bacterial infection or bacteremia (28,58). Within the year after implementation of more "aggressive" iron repletion protocols, two studies did not report a substantial increase in the incidence of infectious complications compared to the year prior to protocol implementation (14,74). In a six-week observational extension of the DRIVE study (DRIVE-II), the ferric gluconate group had a lower number of infections classified as serious than the control group during the 12-week period encompassed by DRIVE and DRIVE-II (10).

In a phase IV study of hemodialysis patients receiving replacement or maintenance iron sucrose, the rate of mortality from infection or sepsis was not significantly different from that observed in the United States Renal Data System (USRDS), and the rate of hospitalization from infection was significantly lower than that of the USRDS (75). Among hemodialysis patients with serum ferritin >800 ng/mL and transferrin saturation <25%, there was no significant difference in incident infection or infectious hospitalization over a 4 month follow-up period between groups suspected and not suspected of having functional iron deficiency; the former group had a higher 3-month mean cumulative intravenous ferric gluconate dose than the latter group (8). Finally, there was no significant difference in the proportion with infection, pneumonia, or cellulitis/ carbuncle comparing patients with intravenous iron dose >455 and ≤455 mg/month (12).

Observational studies evaluating the association between iron use and infection have primarily had a cohort design in which confounding between subjects may not have been adequately controlled. Some studies were primarily descriptive with respect to the association between iron and infection and did not include multivariate analysis (8,10,12,14,59,69,71,72,74,75). Even in studies in which several comorbidities were included in the multivariate models, the

possibility of residual confounding remains (15,26,27,67,68). In the studies that did not find a substantial increase in infectious complications after implementation of an aggressive iron repletion protocol, it is possible that other temporal trends prevented detection of an increased incidence of infectious complications (14,74). As noted by the study authors, comparison of the rates of hospitalization and death from infection between the likely healthier patients in the phase IV study and the general dialysis population is limited by selection bias, although all of the study patients were exposed to intravenous iron compared to only 55–60% during the course of a year in the general dialysis population (75).

Considering the studies of the association between iron usage and infection as a group, some may deserve more emphasis than others. The sample size was relatively small (<150) in many studies (7,8,10,14,28,58,59,66,69,72–74), and the duration of follow-up time was brief in a some studies (7,10,72), which may have limited detection of an effect of iron on infectious outcomes. Of note, most of the large studies did show an association between iron and infection, which may reflect a real effect or residual confounding. Nearly two-thirds (fifteen of twenty-four) of the studies involved hemodialysis patients from countries other than the U.S. (11,26,27,59,69,74) with different anemia management practices (76) or older cohorts (i.e., 2002 or older) of U.S. patients (28,58,60,66–68,70,71,73), limiting generalizability of the results within the setting of current intravenous iron prescription practices for U.S. hemodialysis patients. However, the percentage of studies that found an association between iron and infection risk was the same (50%) in studies conducted inside and outside of the U.S. It is possible that iron sucrose, the most widely used preparation in the U.S. (77), may exert a different effect on infection risk than ferric gluconate or iron dextran based on in vitro data (39,78) and clinical studies (9,73).

Conclusions

The majority of studies focusing on serum ferritin showed an association with infection, but the results from studies evaluating iron usage were more mixed. Overall, the current body of literature appears to favor an association between iron and infection in hemodialysis patients, though several limitations must be acknowledged and publication bias cannot be ruled out. There is a suggestion of possible increased infection risk with iron sucrose compared to ferric

gluconate and iron dextran (9,73), though this must be investigated further in larger studies. Only one study compared the infection risk between bolus and maintenance dosing using multivariate analysis (15), and the finding of higher risk with bolus dosing must be confirmed in future studies before definitive conclusions can be made about which dosing strategy minimizes infection risk. Although there are no clinical data specifically supporting the recommendation by KDIGO and other international CKD anemia guidelines to avoid or withhold intravenous iron among patients with systemic infection (18,20), it may be prudent to do so among most hemodialysis patients with signs or symptoms of infection given that the overall evidence supports an association between iron and incident infectious outcomes. However, it is unclear when iron therapy should be restarted. It is biologically plausible that intravenous iron could exert a relatively acute immunosuppressive effect (37,38), but no clinical studies have specifically evaluated the duration of infection risk posed by iron in hemodialysis patients.

Intravenous iron is important in the treatment of anemia of ESRD, but the optimal strategy to prevent iron deficiency and minimize risk of infection has yet to be identified. It will be important to focus on conducting large studies that compare current dosing strategies and newer agents, consider novel designs to address confounding given that large randomized controlled trials are unlikely, and examine more recent data in the post-bundle era.