Iron Localization and Infectious Disease in Chronic Kidney Disease Patients

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Introduction
Infection is the second leading cause of mortality among patients on maintenance dialysis therapy in Japan and the United States [1, 2]. Recently, the mortality rate from infectious disease was established to be noticeably higher in dialysis patients than in the general population, based on data reported by the Japanese Society for Dialysis Therapy and the National Vital Statistics data from 2008 to 2009 [3]. Amazingly, the standardized mortality ratio from all infectious diseases among dialysis patients compared with that of the general population in Japan was 7.5 (95% CI 7.3–7.6). Among dialysis patients, the types of infectious diseases with significantly higher standardized mortality rates were, in decreasing order, sepsis, peritonitis, influenza, tuberculosis, and pneumonia. Robust associations between lower eGFRs and infection were also observed in other studies [4–6]. A recent meta-analysis showed that the adjusted-rate ratio of tuberculosis in chronic kidney disease (CKD) patients compared with that of the general population was 3.62 (95% CI 1.79–7.33) [7].

Although several factors contribute to higher infection rates in patients with CKD, including those on dialysis therapy, iron metabolism should be considered in the pathogenesis of infectious disease in these patients because iron is indispensable for the proliferation of most human pathogens, which are microorganisms [8]. The role of iron availability in tuberculosis infections has been documented in many reports, including one by the French physician Armand Trousseau in the nineteenth century [9–11].
Most patients on dialysis have renal anemia and are treated with erythropoiesis-stimulating agents (ESA) [1]. The optimal hematopoietic response to ESA requires an adequate supply of iron. These patients can easily develop iron overload when iron supplementation exceeds what is necessary for sufficient erythropoiesis and overwhelms the body’s limited ability to eliminate iron through gastrointestinal loss and procedure-related blood loss [12].

Therefore, it is reasonable to suspect that iron overload in the host can lead to sufficient iron availability for the pathogens and thus promote the development of infectious disease among patients on dialysis, even if their rate of contact with microorganisms is similar to that of the general population.

This review is intended to provide information regarding iron metabolism and infection in CKD patients, as these patients typically have dysregulated iron metabolism [13].

**Host Factors**

Dialysis sessions require the passing of a needle through the skin into an arteriovenous fistula, which present a clear cause of bacterial infections. Especially, buttonhole cannulation has been demonstrated to be associated with higher rates of infectious events than rope-ladder cannulation [14]. This procedure generates an entry point for microorganisms (especially staphylococci) into the bloodstream.

In a prospective cohort, it was demonstrated that iron overload, a previous history of bacterial infection, and the use of dialysis catheters are significant and independent risk factors for bacterial infection in hemodialysis patients, irrespective of any patient-related factors, such as age or time elapsed since the start of dialysis [15].

In an in vitro study, Parkkinen et al. [16] demonstrated that the serum from the patients on maintenance hemodialysis (MHD) without iron supplementation was able to resist the growth of a multiple drug-resistant *Staphylococcus epidermidis* strain. In contrast, in the serum obtained after IV iron administration, bacterial growth was observed in association with an increase in transferrin saturation values and formation of non-transferrin bound iron. When iron-free apotransferrin was added to the serum samples, the serum’s ability to resist the growth was restored in association with the decrease in the transferrin saturation value.

Regarding host defense, several studies have demonstrated the association between iron overload and an impairment of the host’s innate immune response in terms of the T cell and polymorphonuclear leukocyte (PMNL) response, which limits the host’s ability to properly eliminate bacteria and inhibit infection. In terms of peripheral blood leukocytes, therapeutic concentrations of iron in media diminished CD4+ lymphocyte survival through the intracellular oxidative stress caused by iron, which leads to apoptosis [17]. Deicher et al. [18] demonstrated that high-dose parenteral iron impaired the intracellular killing of *S. aureus* by PMNLs. In addition, high doses of IV iron impair the phagocytic activity and diminish the hydrogen peroxide production capacity and microbial killing capability of PMNLs [19]. In a recent in vitro study, iron sucrose treatment led to impaired phagocytic function and increased apoptosis of PMNLs [20].

A consistent finding in patients on hemodialysis is a marked increase in serum levels of proinflammatory cytokines, such as IL-6 and TNF-α. We have demonstrated that in dialysis patients without infection or malignancy, the iron level of PMNLs is increased and is associated with the downregulation of the iron export protein ferroportin (FPN) and upregulation of the iron import protein transferrin receptor (TfR). Additionally, the chemotactic peptide f-Met-Leu-Phe-stimulated degranulation activity of lactoferrin is also decreased [21, 22]. Thus, human hosts with micro-inflammation, such as CKD patients, may use iron withholding as a form of nonspecific immunity to prevent infection in the presence of extracellular-residing microorganisms, even in the absence of infection. Iron sequestration can occur in most cells, including macrophages (fig. 1). However, iron sequestration is involved in the pathogenesis of chronic anemia or functional iron deficiency. When iron is administered to treat a functional iron deficiency, the resulting iron level may exceed the optimal level for erythropoiesis, allowing certain microorganisms to proliferate [13, 19, 23].

Iron sequestration also involves hepcidin, which is believed to be the master regulator of iron homeostasis in vertebrates [24–26]. Hepcidin reduces levels of the only known iron exporter, FPN, resulting in impaired iron recycling by macrophages and a decrease in the plasma iron level. Hepcidin has been found to be highly induced during inflammation, especially by IL-6, and by iron. However, we previously demonstrated that the serum hepcidin level can be exclusively associated with the ferritin level in patients on MHD in the absence of apparent inflammation and independent of the levels of inflammatory cytokines [27]. Several reports have confirmed these observations [28–30]. These data suggest that excessive iron storage or administration increases hepcidin levels and accelerates iron sequestration in most of the cells in the body (fig. 1).
Host–Pathogen Interactions

The survival and replication of microorganisms rely on the acquisition of various host compounds, such as lipids and amino acids. In addition, most microorganisms depend on iron to some extent [8]. This appears to be true for most human pathogens, which have evolved an array of mechanisms to compete with the host’s evolution of iron-withholding strategies. These strategies include the production of siderophores (high-affinity iron-chelating compounds), which capture and gather iron from the host’s supply of transferrin-bound iron [31]. The beneficial effects of iron chelators for control of microorganisms have been observed in several experimental infection systems [32–34].

Above all, the encounter of microbial pathogens with iron could be a significant determinant of infectious disease severity. Iron localization in the intracellular or extracellular spaces of the human body is modulated by many factors, specifically iron transport proteins, which are regulated by hepcidin and proinflammatory cytokines. Furthermore, pathogenic bacteria can be classified into 2 categories, intracellular and extracellular, according to their invasive properties [35]. In the human body, some bacteria mainly proliferate in the extracellular environment, while others mainly proliferate in the intracellular environment. However, it is sometimes hard to discriminate between intracellular and extracellular pathogens because these terms are vague when used in the context of microbial life. For example, most, if not all, intracellular pathogens must survive in the extracellular space prior to entering the cell or after leaving it, while at least one pathogen that has not conventionally been recognized as intracellular pathogen, S. aureus, can enter and survive within a wide variety of mammalian cells [35, 36].

Thus, altered iron availability in the intracellular and extracellular environments caused by an increase or a decrease in the expression of hepcidin and proinflammatory cytokines may play a major role in the outcome of host-pathogen interactions (fig. 2). As pathogenic agents, all bacteria can exist in an infective state or in a rapid growth state, and the difference between these 2 states may determine the virulence of the infection. Most microorganisms are killed by the host’s immune cells through processes that usually involve phagocytosis and lysosomal disruption. However, some organisms, including Yersinia, Salmonella, Listeria, Shigella, Legionella, and Mycobacteri-
um species, are capable of growing inside macrophages and avoiding destruction [37]. Thus, the host’s redistribution of iron as a defense against extracellular organisms or infection may increase its susceptibility to the deleterious effects of intracellular organisms [8]. For microbes that mainly proliferate in the extracellular environment, the host’s strategy for iron sequestration in cells may efficiently suppress the microbes’ proliferation. Moreover, intracellular microbes may gain the ability to make the inside cells survive by acquiring a sufficient amount of iron. In mouse model of Salmonella typhimurium infection, it was demonstrated that bacteria stimulated the expression of host hepcidin through the estrogen-related receptor γ [38]. In line with these observations, emerging data support a role of hepcidin in the pathogenesis of a number of infections, especially those caused by intracellular pathogens [39]. On the other hand, hepcidin-induced hypoferrremia has been shown to favor the control of Vibrio vulnificus growth for a host defense [40]. Thus, hepcidin-induced iron sequestration may increase the pathogenesis of some intracellular organisms, but it is also protective against other extracellular organisms.

Mycobacterium tuberculosis is a representative intracellular pathogen that thrives inside host macrophages and other cell types. It resides in a membrane-bound vacuole, the phagosome, and can also escape into the cytosol at late stages of infection [41]. A key feature of M. tuberculosis is its ability to obtain metal cations and manipulate metal cation trafficking inside infected macrophages. The relationship between the intracellular iron of macrophages or hepcidin and the growth of M. tuberculosis was well illustrated in peripheral blood mononuclear cells from hereditary hemochromatosis patients with mutations in HFE, who should have lower hepcidin levels [42, 43]. M. tuberculosis growth and iron content were reduced in the macrophages from these patients compared with those from healthy adult volunteers [42]. As for the host defense, essential micronutrients, such as iron, are

![Fig. 2. Relationship between iron availability and the virulence of extra- and intra-cellular pathogens. In the presence of hepcidin or proinflammatory cytokines (e.g. TNF-α), iron is sequestered in the cells, making more iron available to intracellular pathogens. In the absence of hepcidin and TNF-α, iron remains in the extracellular environment and this may promote the growth of extracellular pathogens. Cytokines = Proinflammatory cytokines.](image-url)
sequestered from intracellular *M. tuberculosis* by being exported from the phagosome by the divalent metal cation transporter natural resistance-associated macrophage protein 1 (Nramp1) [22, 41, 44] (fig. 1). It has been demonstrated that mutations in Nramp1 cause susceptibility to infection with the bacteria *Salmonella* and *Mycobacteria* and the protozoan *Leishmania*, which are classified as intracellular pathogens [45]. However, we demonstrated that the expression level of the phagosomal iron transport protein Nramp1 was significantly decreased in PMNLs from dialysis patients, and TNF-α caused a decline in the expression level of this protein in control PMNLs [13, 22]. Thus, the iron storage pool in the phagosomes of infected macrophages may also favor the proliferation of *Mycobacterium* in CKD patients.

**Relationship between Iron and Infection in Clinical Situations**

An association between iron and infection is still theoretically expected because iron promotes bacterial growth and impairs host immunity. However, the effects of iron stores and iron therapy on infectious disease are still controversial to date [46].

A meta-analysis of randomized controlled trials evaluating the effect of IV iron use (often administered as frequent boluses) on the risk of infection demonstrated that IV iron is associated with a 30% greater risk of infection compared with oral iron and no iron therapy [47]. However, this analysis was not in CKD patients, and also criticized for the possibility of unmeasured bias. In addition, in a retrospective cohort study of 117,050 prevalent hemodialysis patients, the administration of large boluses of IV iron to address iron deficiency was shown to be associated with increased infection-related hospitalization and death compared with the administration of smaller doses of IV iron maintenance therapy [48].

In our observational cohort study of dialysis patients, we also demonstrated that patients with high serum ferritin levels (≥100 ng/ml) or large ferritin level fluctuations are at an increased risk of all adverse events, including infection, compared with patients with lower serum ferritin levels (<100 ng/ml). Notably, the risk of all adverse events is significantly higher among patients who are treated with oral or IV iron compared with those who are not treated with iron [49]. However, these cut-off ferritin thresholds have been observed only in the Japanese population so far.

A recent study showed that in patients on dialysis, dose accumulations <1,050 mg of IV iron over 3 months and <2,100 mg over 6 months are not associated with all-cause, CV, or infection-related mortality [50]. Furthermore, a retrospective observational cohort study using data from the US Renal Data System showed that in patients who had received IV iron in the 14 days preceding their first hospitalization for bacterial infection, the administration of IV iron was not associated with higher mortality compared with no iron administration [51].

Even when the main outcome measure was patient survival, studies on the effect of iron on mortality rate in nonconcurrent cohorts by the same research group spanning several years had conflicting results. In the first analysis of 10,169 patients on MHD who had received >1,000 mg of IV iron over a 6-month period, significantly higher mortality was found compared with the reference group, which received <1,000 mg of IV iron during the same period [52]. Another large multicenter study with a similar cohort showed no significant association between any level of iron administration and mortality using another analysis including lagged time-dependent models [53].

There are also recent conflicting reports of randomized controlled trials about the association between iron therapy and infection. In patients with iron-deficiency anemia and non-dialysis-dependent CKD (FIND-CKD study), the 12-month efficacy and safety of IV ferric carboxymaltose (FCM) were assessed compared with oral iron. The incidence of infections was similar between treatment groups (high-ferritin FCM, low-ferritin FCM and oral iron groups) [54]. On the other hand, another randomized controlled trial showed that in stages 3 and 4 CKD patients, the incidence of lung and skin infections was increased 3- to 4-fold in the IV iron group compared with the oral iron group [55]. In this study, however, the higher incidence of infections was related to a greater number of infections in the same patients.

The KDIGO Controversies Conference on Iron Management in CKD was convened to gather a global panel of multidisciplinary clinical and scientific expertise to identify key issues relevant to the optimal iron management in CKD [56]. They concluded that present available data did not allow any firm statement to be made on the potential dangers of high-dose iron administration and high ferritin levels, and concluded that RCTs are urgently required to address the shortfall in the evidence base, as only randomized controlled trials can differentiate cause and effect.

Thus, the results from studies evaluating the association between iron usage or storage and infection rate were more mixed. In considering the relationship between iron administration or storage and the prevalence of in-
fection, we should consider many factors such as the baseline ferritin level, iron preparation type, iron dosing pattern, routes of iron administration, and duration of intervention [57]. In addition, the pathogen proliferation site, extracellular or intracellular, may determine the degree of virulence of infectious disease. In patients with higher ferritin levels, the hepcidin levels should be increased to cause the maximal sequestration of iron in cells. Regarding the routes of iron administration, the pattern of the increase in the transferrin saturation rate when iron is administered intravenously may differ from the pattern resulting from oral iron administration [58–60]. High transferrin saturation rates can affect the level of non-transferrin-bound iron, which may favor the growth of extracellular pathogens [59, 61, 62].

Management of Vulnerability to Infection

Two possible mechanisms causing susceptibility to infection in CKD patients should be managed: (1) the prevention of excessive iron administration for the treatment of renal anemia and (2) the attenuation of inflammation associated with dysregulated iron metabolism.

Following randomized clinical trials, which include normal hematocrit study, CREATE study, CHOIR study and TREAT study, the issue that is of concern is that higher hemoglobin targets and/or higher ESA doses may cause significant toxicity and an increase in iron dosing has occurred [63]. For clarifying the optimal iron therapy for patients with CKD, which prevents infection and cardiovascular events, large multicenter randomized controlled trials designed to evaluate the long-term safety of iron administration regimens are required. Researcher of FIND-CKD is currently examining a major intravenous iron outcomes study in the United Kingdom. This multicenter study – producing efficient erythropoiesis in CKD patients. Following randomized clinical trials, which include normal hematocrit study, CREATE study, CHOIR study and TREAT study, the issue that is of concern is that higher hemoglobin targets and/or higher ESA doses may cause significant toxicity and an increase in iron dosing has occurred [63]. For clarifying the optimal iron therapy for patients with CKD, which prevents infection and cardiovascular events, large multicenter randomized controlled trials designed to evaluate the long-term safety of iron administration regimens are required. Researcher of FIND-CKD is currently examining a major intravenous iron outcomes study in the United Kingdom. This multicenter study – producing efficient erythropoiesis in CKD patients.

On the other hand, the appropriate usage of ESA may be effective for the attenuation of iron-induced oxidative stress, because ESA could promote the consumption of iron for erythropoiesis, decrease hepcidin level through erythrophorene synthesis and release sequestered iron from storage sites [64]. However, the effect of ESA on infection in association with iron metabolism has not been well clarified yet. Among ESAs, the new erythropoietic strategy, hypoxia-inducible factor (HIF) stabilizers – has now emerged. An increase in HIF has been expected to boost diverse myeloid cell antimicrobial activities and reinforce the bactericidal capacity of immune cells [65]. Therefore, clinical trials are needed to investigate whether drugs that upregulate HIF have an impact on the frequency of bacterial infection during the correction of anemia.

Conclusion

In conclusion, we showed an increased infection rate in dialysis patients compared with that in the general population. The link between greater iron use or storage and infection is still theoretically expected, but is still controversial to date. The long-term safety of iron-prescribing practices should be established to improve the survival of patients on dialysis, as iron is essential for erythropoiesis but also activates the growth of bacteria. Future randomized prospective controlled studies are needed to address this important issue and determine the preparation, administration routes and doses of iron that allow a lower infection rate while still producing efficient erythropoiesis in CKD patients.

Disclosure Statement

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