Iron Management in Chronic Kidney Disease: Report from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference

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ABSTRACT

Before the introduction of erythropoiesis-stimulating agents (ESAs) in 1989, repeated transfusions given to patients with end-stage renal disease caused iron overload, and the need for supplemental iron was rare. However, with the widespread introduction of ESAs, it was recognized that supplemental iron was necessary to optimize hemoglobin response and allow reduction of the ESA dose for economic reasons and recent concerns about ESA safety. Iron supplementation was also found to be more efficacious via intravenous compared to oral administration, and the use of intravenous iron has escalated in recent years. The safety of various iron compounds has been of theoretical concern due to their potential to induce iron overload, oxidative stress, hypersensitivity reactions and a permissive environment for infectious processes. Therefore, an expert group was convened to assess the benefits and risks of parenteral iron, and to provide strategies for its optimal use while mitigating the risk for acute reactions and other adverse effects.

BACKGROUND

Iron is a vital element for numerous bodily functions, most notably as an ingredient of haemoglobin (Hb), the major oxygen-carrying protein in the body. Most healthy persons can achieve a stable iron balance, managing to ingest the required amount of iron in the diet to compensate for the small amount of daily iron losses from the gut. However, in many patients with advanced chronic kidney disease (CKD) are in negative iron balance as a result of reduced dietary intake, impaired absorption from the gut, and increased iron losses. This is particularly true in patients receiving hemodialysis (HD) for whom supplemental iron is often essential to keep pace with blood loss and the requirements for erythropoiesis. For many decades, it has been recognised that oral iron supplementation is unable to maintain optimal erythropoiesis, and intravenous (IV) iron has become an important component in anemia management in this patient population, particularly since the introduction of erythropoietin therapy in the late 1980s.

Intravenous iron is a highly effective means of replacing iron deficits and can enhance erythropoiesis, allowing lower requirements for erythropoiesis-stimulating agent (ESA) therapy. This is particularly important since the realization that ESA therapy may result in a number of adverse clinical outcomes, most notably stroke, venous thromboembolic disease and vascular access thrombosis in a variety of populations. Furthermore, healthcare reforms in the US involving bundled payments for dialysis in which the cost of ESA therapy is included have prompted many nephrologists to increase the amount of IV iron administered to patients on dialysis, in a concerted effort to avoid iron deficiency and optimize erythropoiesis while minimizing ESA dose requirements. Aside from changes in laboratory parameters, the evidence base evaluating outcomes related to the use of IV iron is sparse and the effect of IV iron on hard clinical outcomes including death and major health events is uncertain. Moreover, there is evidence from laboratory, animal, and observational studies that IV iron may exacerbate oxidative stress, potentiate atherogenesis and cardiovascular (CV) toxicity, and increase the propensity for infections, as well as occasionally induce hypersensitivity reactions.

This conference was convened to critically examine the evidence base, and to identify gaps in knowledge so as to direct future clinical research. Four main themes were discussed: iron overload, oxidative stress, infections, and hypersensitivity reactions.

ACHIEVING THE RIGHT BALANCE: IRON DEFICIENCY VS. IRON OVERLOAD

Causes of iron deficiency

Patients with CKD are prone to iron deficiency, and its etiology is multifactorial. The definition of iron deficiency can be considered under two main categories, namely ***absolute*:** when there is a deficiency of total body iron stores, and ***functional*:** when there is ample or increased total body iron stores, but with sequestration of iron in the reticuloendothelial system (RES). Thus, inadequate iron supply for erythropoiesis can also commonly occur in the absence of absolute iron deficiency.

Absolute iron deficiency is caused by:

- Blood loss for laboratory tests, aggravated by hospitalizations
- Gastrointestinal (GI) losses (may be exacerbated by systemic anticoagulation during dialysis, and/or the use of maintenance oral anticoagulants or anti-platelet drugs used for the treatment or prevention of cardiovascular disease [CVD])
- Blood losses associated with the HD procedure, including dialyzer blood loss and blood loss from the arteriovenous fistula or graft puncture site and from catheters
- Reduced intestinal iron absorption, at least in part due to increased hepcidin levels, and medications (e.g., proton pump inhibitors and calcium-containing phosphate binders)[1-3]
- Reduced intake due to poor appetite, malnutrition, and dietary advice (e.g., protein restriction)

With respect to functional iron deficiency, sequestration of iron within the RES is primarily due to inflammation. Since transferrin is a negative acute phase protein (i.e., levels drop sharply in response to inflammation), serum transferrin concentrations tend to be reduced in patients with CKD.[4] As a result, total iron binding capacity is decreased. At a given transferrin saturation (TSAT), the absolute amount of iron bound to transferrin in the circulation and available for erythropoiesis is thus lower in patients with CKD than in healthy persons with normal or near normal kidney function. Stimulation of erythropoiesis with ESAs creates an increased demand for iron and can unmask and/or aggravate decreased iron availability.

Iron loss is largely due to blood loss. The relation between blood loss and iron loss depends on the Hb level (e.g., Hb 12 g/dl: 0.40 mg iron/ml blood; Hb 10 g/dl: 0.36 mg iron/ml blood). In patients with non-dialysis-requiring CKD, the average GI blood loss can be elevated (estimated blood loss of 3.2 ml/d, approx. 1.2 l/year, corresponding to about 0.4 g iron/year) as compared to healthy persons (0.83 ml/d, corresponding to about 0.1 g iron/year).[5] In patients receiving HD, some evidence indicates an even larger increase of GI blood loss (mean 5.0 ml/d).[6] Procedure and laboratory test-related blood loss of patients on HD is of the order of 2-5 l/ year,[7] but may

vary considerably over time and among patients; blood loss is also influenced, for example, by anticoagulant and antiplatelet agent prescription.[8-10]

An alternative approach to estimate annual iron losses is to define the amount of IV iron administered to patients receiving HD to maintain Hb, TSAT and ferritin constant.[4, 11, 12] The validity of this approach depends on the assumption that under such conditions the amount of iron administered equals iron losses. Such studies revealed that after initial iron loading to achieve a higher TSAT and Hb level, a maintenance IV iron protocol needed to maintain Hb levels can be established. Though the use of a maintenance iron protocol is associated with lower ESA requirements, two of three studies reported progressively rising ferritin levels over time, suggesting that no equilibrium was achieved. The range of maintenance IV iron differed considerably between 25-150 mg/week and is likely to be a function of the patient's dietary intake, proportion of dietary iron absorbed, and particularly blood losses.

In aggregate, based on the evidence available, iron losses for patients on HD are frequently considered to be of the order of 1-2 g/year, but may be highly variable, and in some patients iron losses may be as high as 4-5 g/year.

Definition of iron deficiency and means of diagnosis

Both ferritin and TSAT have their shortcomings in assessing iron status and guiding iron therapy in patients with CKD.[13-16]

The diagnosis of absolute iron deficiency is usually based on low serum ferritin concentrations (< 20-30 µg/l) which reflect low body iron stores. In patients with CKD, due to the presence of inflammation, threshold values indicating iron deficiency are generally considered to be higher than in those without kidney disease. Serum ferritin concentrations of 100 and 200 µg/l are frequently cited as cutoff values in patients with non-dialysis-requiring and dialysis-dependent CKD, respectively.[17]

The TSAT (%) is the serum iron x 100 divided by the total iron binding capacity (TIBC). The TIBC reflects transferrin, the protein to which virtually all iron in the blood is bound. Although the evidence is rather limited, it is generally felt that a transferrin saturation < 20% is indicative of absolute iron deficiency, although transferrin saturations above this do not exclude this condition.[17]

Even when iron stores and circulating iron are sufficient, iron supply for erythropoiesis can be inadequate as in instances during intense stimulation of erythropoiesis with ESAs, or under conditions of blocked iron release from macrophages by inflammation.

The measurement of soluble transferrin receptor (sTfR) in serum has also been proposed as an indicator of adequate iron supply to the erythron. However, sTfR is of less informative value in patients on ESA because increased erythropoiesis itself raises sTfR levels. Its clinical use is also hampered by limited availability and insufficient standardization of methods in clinical laboratories. There are also few published studies describing the use of sTfR in patients with CKD.

A higher percentage of hypochromic red cells (> 6-10%) and a lower reticulocyte Hb content (CHr < 26-30 pg) as currently measured on specific automated cell counters (e.g., Technicon H*3, Siemens ADVIA 120) are the best indicators of inadequate iron supply and correlate with a reduced response to IV iron and higher ESA requirements.[11, 16, 18-22] Both percentage of hypochromic red cells and CHr can be performed simultaneously during routine blood counts with minimum or relatively low incremental costs and no additional blood sampling. The advantage of the Hb content of the reticulocytes (CHr) is that it is a relatively fast response marker of iron status, reflecting the iron status that existed 3-4 days before. The percentage of hypochromic cells identifies changes in iron delivery to the erythrocyte precursor cells less rapidly, since it takes time for the change to be seen in a larger and older population of cells. Moreover, since the percentage of hypochromic red cells is dependent on the size of the red blood cells, it is strongly influenced by the time between sampling and analysis; it can perform well in the research setting (and indeed may be the best indicator in this context[16]), but is not as helpful in the 'real-life' clinical setting and it is therefore not recommended by the KDIGO Anemia guideline. Similar parameters are also available on more recently marketed hematology analyzers (e.g., Abbott, Sysmex, and Beckman Coulter). Preliminary studies indicate that the clinical utility of these newer parameters may be comparable to those from the Technicon H*3 and ADVIA 120 analyzers, but their numerical values differ because of differences in technology.[23, 24] Data on red cell and reticulocyte parameters from these newer analyzers in patients with CKD are lacking.

Assessment of hepcidin in patients with CKD has augmented our understanding of the pathophysiology of inadequate iron supply for erythropoiesis. Measuring serum hepcidin has been proposed as a means of identifying patients who might benefit from increasing either ESA or IV iron dosing,[25] but to date, such approach has not been shown to be clinically useful.[26-29] Furthermore, measurement of the bioactive hepcidin-25 in CKD is heavily dependent on the methodology used. Hepcidin assays are not harmonized or standardized and in contrast to mass spectrometry based assays- many of the immunoassays cross-react with biologically inactive hepcidin isomers which accumulate in CKD.[30-32] The role of hepcidin as a predictor for progression of anemia in patients with non-dialysis-requiring CKD[33] and CV events warrants further investigation.[34, 35]

Doses of iron required to correct iron deficiency

Since the true amount of iron loss in individual patients and patient groups is uncertain, the precise doses required to compensate for this loss inevitably remain uncertain. Applying doses of IV iron in excess of ongoing losses will result in positive iron balance, the consequences of which are unknown.

In general, IV iron doses in excess of 3 g/year are likely to be associated with an increased risk of exceeding the ongoing iron loss and inducing positive iron balance. In patients who routinely receive IV iron, higher requirements for IV iron to maintain Hb concentrations within a target range, or within the patients' usual range should prompt the search for increased losses, particularly from the GI tract.

Oral iron is generally considered to be associated with a lower risk for iron overload because of the body's ability to regulate intestinal iron absorption through the action of hepcidin and other factors.[36-40] Ferric citrate, a novel oral iron-containing phosphate binding agent, was recently shown to increase serum iron parameters to levels within KDIGO Anemia guideline recommendations.[17, 41, 42] Furthermore, agents under development that potentially interfere with mechanisms controlling enteric iron absorption, such as hepcidin-antagonists,[43] or prolyl hydroxylase-inhibitors,[44] may increase oral iron absorption. The contribution of orally administered iron to total iron balance and the associated risk of iron overload in patients administered with such agents will need to be examined carefully.

Novel developments in iron administration also include the addition of iron in the form of soluble ferric pyrophosphate to the dialysate. This iron formulation has been reported to be directly transferred to circulating transferrin, which may reduce ESA and IV iron dose requirements. In fact, iron administered via this route has been shown to maintain iron balance (i.e., Hb, TSAT, ferritin and ESA dose) at lower doses than previously required with IV administration.[45]

Iron overload

There is no feasible method available to determine total body iron content that would allow a systematic assessment of body iron content in patients with non-dialysis-requiring and dialysis-dependent CKD and as such, a statistical approach to defining increased or decreased body iron content cannot be currently ascertained. Even if it were possible to accurately quantify total body iron content, it is unclear whether it would be possible to ascertain a specific level toxic to tissues and organs. The body has a physiological means of storing iron in cells of the reticuloendothelial system, primarily tissue macrophages, such as Kupffer cells in the liver. Levels beyond which harm occurs would be extremely difficult to elucidate and may even differ from one individual to

another. A published methodology determining the ratio of sTfR to serum ferritin is probably not reliable in the presence of inflammation and has not been validated in patients with CKD.[46] Thus, the present definitions of iron deficiency and overload remain imperfect and one has to rely on presumed functional consequences of decreased or increased iron stores and surrogate markers.

Iron overload represents a condition of increased total body iron content that is possibly associated with a time-dependent risk of organ dysfunction. Pathological iron overload represents a condition of increased body iron content associated with signs of organ dysfunction that are presumably caused by excess iron.

The consequences of increased body iron content depend on a variety of factors, including the distribution of iron among parenchymal cells and cells of the RES, the duration of iron excess in relation to the life-expectancy of the patient, co-morbidities and others. The circumstances under which increased iron content is associated with clinically relevant adverse consequences and the nature of these consequences are insufficiently defined.

Indirect evidence, including experience in patients with inherited hemochromatosis suggests that parenchymal iron excess and labile iron can be harmful, whereas iron stored within cells of the RES may be of less concern,[47, 48] although intrahepatic iron might induce hepatic damage through iron-induced mesenchymal activation.[49]

Serum ferritin concentrations, when elevated, do not always correlate with elevations in liver iron content.[50-52] Hyperferritinemia is thus not synonymous with iron overload. In addition, the level of serum ferritin does not indicate whether iron is stored in parenchymal cells or cells of the RES,[53] although animal data suggest that macrophages contribute significantly to serum ferritin concentrations.[54]

High transferrin saturation facilitates parenchymal iron deposition. Of particular concern appears to be a combination of high TSAT and high serum ferritin concentrations based on experience in patients with hereditary hemochromatosis[55] and transfusion-induced iron overload.[56]

In patients in whom transferrin becomes highly saturated, additional iron is released into the circulation and is bound to lower molecular weight compounds. This plasma non-transferrin bound iron (NTBI) and its labile (redox active) component (LPI) are potentially toxic forms of iron that contribute to oxidant–mediated cellular injury. Routine clinical measurement of NTBI and LPI levels is limited by insufficient harmonization and standardization of methods and a paucity of data on clinical correlations.[56-58]

Magnetic resonance imaging (MRI) scans using specific protocols have been shown to provide a reliable estimate of tissue iron content in non-CKD populations.[59, 60] Measurements in unselected patients receiving HD suggest that liver iron content is increased compared to reference values in the majority of patients and is substantially increased in approximately one-third of patients.[61] These data confirm those obtained by superconducting quantum interference devices (SQUID) technology[62], another non-invasive technique for determining tissue iron content that is both costly and limited in availability, and MRI on patients receiving IV iron treatment.[50-52] Liver iron content as estimated by these technologies has not consistently been found to correlate with serum ferritin concentrations,[50-52] but was directly associated with previous iron dosing. However, the clinical relevance of increased liver iron content in the absence of elevated liver enzymes is unclear. Another study in a selected HD patient population with high TSAT and serum ferritin concentrations has also provided evidence for excess pancreatic iron accumulation.[52]

The MRI technique should be used in further research to better understand the value of detecting clinically relevant changes in tissue iron content. It is not clear, for example, whether an increased iron signal from the liver on MRI represents iron uptake in the Kupffer cells of the RES (which are thought to be physiologically better designed to cope with iron loading and hence iron deposition) or in hepatocytes of the liver parenchyma; iron deposition in these distinct systems may have different physiological and clinical relevance. Comparison of signals in spleen and liver may provide information about iron distribution between parenchymal cells (liver) and RES (spleen). At present, there is insufficient evidence to support the routine use of hepatic MRI in guiding iron therapy in clinical practice.

Impact of iron overload on organ function and patient outcomes

Iron can have beneficial and adverse effects on organ function. Organ toxicity associated with iron overload in hematological diseases depends on various factors, including the magnitude and speed of iron accumulation. The main target organs are liver, myocardium, endocrine glands, and joints.[55, 63] However, the magnitude, distribution and duration of iron accumulation in CKD patients may be insufficient to produce toxicity similar to that observed in hemochromatosis. Given that IV iron use has increased markedly in HD patients over the past few years,[64, 65] the exposure to higher amounts may not have accrued long enough to detect such toxicity. Although end-organ damage from IV iron administration in patients with kidney disease has not been unequivocally established, at present one cannot exclude the toxicity potential of iron induced by repeated high-dose IV iron administration in CKD. Systematic surveillance through registries may therefore be helpful.

There is a rare form of genetically determined iron accumulation in macrophages that is characterized by very elevated serum ferritin concentrations (> 1000 µg/l) and normal TSAT,

and is not associated with any obvious toxicity, suggesting that parenchymal rather than RES iron deposition is relevant for long-term toxicity.[48]

OXIDATIVE STRESS IN UREMIA

Oxidative stress or oxidant-derived tissue injury results from an overproduction of reactive oxygen/nitrogen species or impairment in the cellular antioxidant enzymatic activities, leading to oxidation of macromolecules such as proteins, carbohydrates, lipids, and DNA. Several surrogate markers of oxidized macromolecules are now available for estimating oxidative stress.[66] For example, lipid oxidation markers include malondialdehyde (MDA), thiobarbituric acid-reactive substances (TBARS), F2-isoprostanes and antibodies against ox-LDL; protein oxidation biomarkers encompass advanced oxidation protein products (AOPP), advanced glycation end products (AGEs) and carbonyls. Meanwhile, marker such as 8-hydroxy 2′-deoxyguanosine (8-OHdG) and the Comet assay are potentially useful in detecting DNA oxidation. Among major practical concerns barring the widespread adoption of these biomarkers in clinical setting are the absence of established reference ranges, use of different analytical techniques, and the lack of knowledge regarding the relations among impaired kidney function and associated co-morbidities on their circulating levels.[67] Thus, at the present time there is no gold standard that can be recommended for measuring or monitoring oxidative stress to guide clinical risk assessment or prognosis. In the future, perhaps a systematic metabolomics approach could be employed to identify biomarkers or constellations of biomarkers to offer insight into specific stimuli of oxidative stress.

Increased levels of oxidative stress markers are present in uremic plasma and are thought to be fingerprints of increased oxidative stress (Figure 1). Oxidative stress occurs early in the evolution of impaired kidney function and is believed to herald a poor prognosis,[68] and often associates with persistent inflammation.[66] Of the four different types of oxidative stress in the uremic milieu: carbonyl stress, classic metal-related oxidative stress, nitrosative stress and chlorinated stress, the last pathway seems to be the most important.[66] A systematic review of clinical trials suggests that several markers have emerged as possible indicators of oxidative stress and antioxidant status.[67]

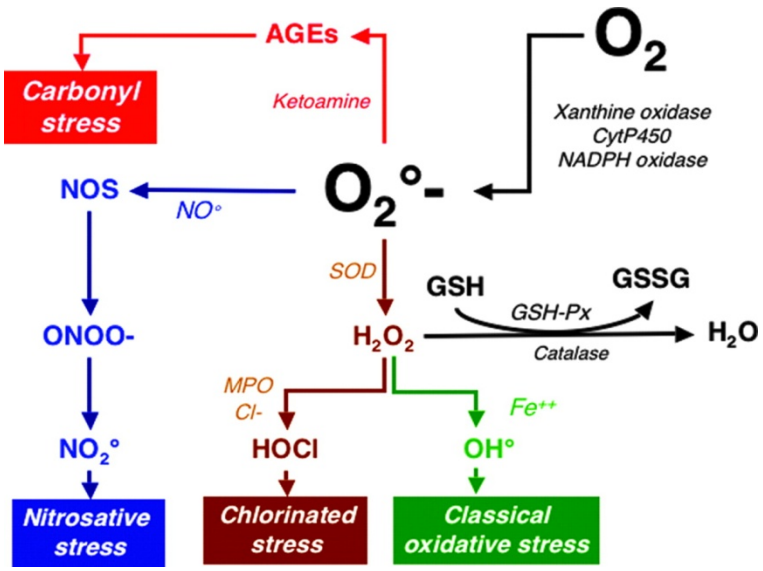

Figure 1. Schematic representation of oxidation and antioxidant pathways in chronic kidney disease. AGEs, advanced glycation products; CytP450, cytochromes P450; GSH, reduced glutathione; GSH-PX, glutathione peroxidase; GSSG, oxidized glutathione; MPO, myeloperoxidase; NADPH, nicotinamide-adenine dinucleotide phosphate; NOS, nitric oxide synthase; $ONOO^-$,peroxynitrite; SOD, superoxide dismutase. Reproduced with permission from Stenvinkel et al.[69]

Role of iron in aggravating oxidative stress and inflammation in CKD

Clinical studies in CKD patients have shown that IV iron administration provokes oxidative damage to peripheral blood lymphocyte DNA,[70] protein oxidation,[71] and lipid peroxidation.[72] A recent meta-analysis based on 34 studies and 2658 patients concluded that IV iron "exerts some effects on markers of oxidative stress of unclear significance."[73] On the other hand, one study showed that increased peroxide concentrations during HD were not associated with IV iron administration since the group of patients randomized to 'no iron' developed a similar transient increase in peroxide levels.[74] Thus, studies are needed to disentangle the putative pro-oxidative effects of repeat iron infusions from multiple other sources of oxidative stress in the uremic milieu, such as retained uremic solutes, hypoxia, hyperglycemia, dyslipidemia, protein energy wasting, inflammation, and the dialysis procedure itself.[68, 75] It will be important for future studies to assess the basal oxidative stress status before IV iron administration in order to evaluate the magnitude and duration of iron-induced oxidative stress.

In addition to direct pro-oxidative effects, studies have shown that administration of IV iron compounds promotes cellular apoptosis[76] endothelial dysfunction[77, 78] and monocyte adhesion.[76, 77] However, one study showed that IV iron reduces the levels of circulating pro-inflammatory

cytokines such as interleukin-1 and TNF in dialysis patients.[79] Whereas it was reported that IV iron increased generation of reactive oxygen species that lead to short-term activation of NF-κB in monocytes[80] and Kupffer cells,[81] another study showed transient activation of anti-oxidant nuclear factor-erythroid-2-related factor 2 (Nrf2) and increased expression of genes for detoxifying and antioxidant molecules,[82] which will with time dampen oxidative stress. Studies are needed to clarify if decreased anti-oxidative defence mechanism(s) in the uremic milieu may prolong and/or increase the magnitude of oxidative stress following iron injections in this vulnerable patient group.[83] Since the available IV iron formulations are structurally heterogenous iron-carbohydrate nanoparticle complexes that exhibit different stability and pharmacokinetic profiles,[84] further research should be conducted to dissect the specific effects of various IV iron compounds on the magnitude and time response of both established and novel oxidative stress biomarkers. Since the expression of Nrf2 is down-regulated in the inflamed and pro-oxidative uremic milieu,[85] detailed studies should elucidate the role of antioxidant defense mechanisms in uremia following IV iron injections.

Iron-mediated oxidative stress and CV risk

Despite numerous basic and clinical studies, the question of whether or not iron administration promotes atherosclerosis and arterial remodeling remains unresolved. Moreover, although iron has been shown to be present in human atherosclerotic plaques[86] it is not yet proven that this accumulation is deleterious and promotes CVD. A recent study in ApoE knockout mice and ApoE/ffe mice fed with a high fat diet demonstrated that the atherosclerotic plaque size was not increased in mice with elevated macrophage iron, in contradiction to the 'iron hypothesis.'[87] In contrast, a recent study in the mouse remnant kidney model showed that iron sucrose aggravated early atherosclerosis by increasing monocyte-endothelial adhesion and increased superoxide production.[88] In a historical cohort of 58,058 patients receiving HD, IV iron doses >400 mg/month were associated with higher CV death rates;[89] however, as with all observational studies, this finding is prone to confounding. Although clinical studies have also demonstrated significant correlations among cumulative iron dose, intimal media thickness[90, 91] and CV events,[92] these findings are again difficult to interpret due to their observational nature and confounding by indication. A recent retrospective study of 117,050 patients showed no association between large doses of iron and short-term CV morbidity and mortality.[93] Prospective controlled studies are needed to clarify if iron promotes atherosclerosis, arterial remodelling, and accelerates CV mortality, especially in vulnerable subgroups such as patients with diabetes mellitus and/or persistent inflammation.

Recent evidence suggests links between iron deficiency/IV iron supplementation and chronic kidney disease-mineral bone disorder (CKD-MBD), a syndrome that includes renal osteodystrophy and extraskeletal calcification with important clinical consequences.[94] In a study of women with iron deficiency due to uterine bleeding, transient, marked and asymptomatic

hypophosphatemia was observed after IV administration of ferric carboxymaltose (FCM).[95] This finding was confirmed in 47 patients with non-dialysis-requiring CKD patients in whom a single injection of 1000 mg FCM induced a reduction in both serum phosphate and FGF23 that persisted for 3 months,[96] while asymptomatic mild reductions in serum phosphate were seen in the high-dose IV iron arm of the FIND-CKD study, also in response to FCM.[97] Since iron deficiency stimulates FGF23 transcription in osteocytes[98] and an inverse relationship between iron administration and serum intact FGF23 concentrations was observed in a dialysis population,[99] it could be speculated that iron therapy might have beneficial effects in the uremic milieu via inhibition of FGF23. A recent study by Wolf et al.[100] in women with iron deficiency due to heavy uterine bleeding showed that whereas iron deficiency increases C-terminal FGF23 levels, FCM treatment (in contrast to iron dextran) temporarily increases intact FGF23 and promotes hypophosphatemia. Additional, carefully controlled studies of the short and long-term effects of various IV iron formulations on CKD-MBD biomarkers are needed.

Recent studies also link iron metabolism with bone disease and vascular calcification. A study on aortic smooth muscle cells demonstrated that the osteoblastic transformation provoked by elevated serum phosphate was diminished by ferritin/ferroxidase activity[101] and another study showed that iron overloading had suppressive effects on vascular calcification in rats;[102] thus, links between iron overload, iron supplementation and vascular calcification merit further attention in clinical studies. The recent observations that a higher serum ferritin is associated with lower bone mineral density in women >45 years in the general population,[103] that iron-provoked inhibition of osteoblast activity is mediated by ferritin and its ferroxidase activity[104] and a cell-culture study showing that chronic iron accumulation decreased bone formation,[105] highlight the importance of understanding the effects of long-term iron supplementation on uremic and non-uremic bone disease.

Increased hepcidin: important mediator of CV risk?

Hepcidin is the key iron regulatory protein synthesized in the liver that is sensitive not only to iron deficiency but is also upregulated in response to increased circulating and stored iron levels,[106] inflammation[107] and infections,[108] and is down-regulated by hepcidin-inhibitors, including testosterone,[109] estrogen,[110] and erythroferrone.[111] Some studies suggest that increased hepcidin may increase CV risk by preventing mobilization of iron from macrophages (Figure 2). Hepcidin and macrophage iron correlate with monocyte chemoattractant protein-1 release and vascular damage in patients with metabolic disease.[112] Moreover, in a clinical study of 766 women without kidney disease, serum hepcidin concentrations were associated with the presence of atherosclerotic plaques.[113] Indirect evidence for a pro-atherogenic role of hepcidin comes from a study that shows that pharmacological suppression of hepcidin increases macrophage reverse cholesterol transport and limit atherosclerosis.[114] In the context of CKD, the evidence that links increased hepcidin to CVD is limited. However, one study showed an association between

increased hepcidin and arterial stiffness in patients receiving HD,[34] and in the CONvective TRAnsport Study (CONTRAST) of 405 patients receiving HD, serum hepcidin-25 was related to CV events even after correction for the presence of inflammation.[35] Since clinical conditions that affect serum hepcidin concentrations (such as inflammation and low testosterone) may have independent pro-atherogenic effects, further research should clarify if hepcidin has independent pro-atherogenic effects in the uremic milieu. Studies of pharmacologic modulation of hepcidin would further elucidate the potential role of hepcidin in arterial remodelling and atherosclerosis.

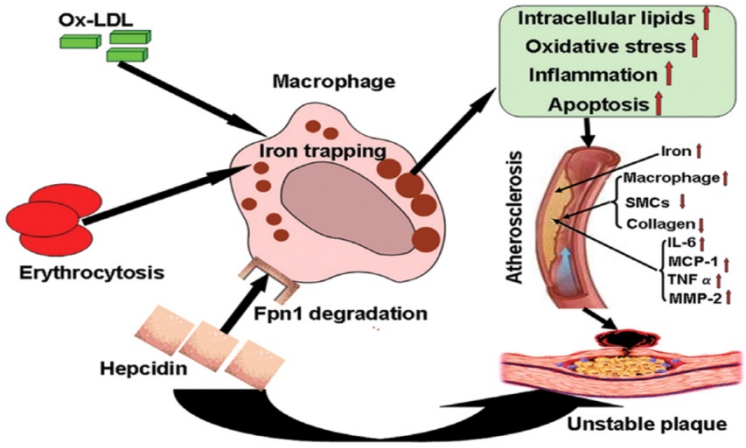

Figure 2. Proposed mechanisms underlying the hepcidin-induced plaque instability. In the setting of erythrophagocytosis, hepcidin suppresses iron release from macrophages via downregulation of iron-exporting protein Fpn1 and increases iron storage. Iron trapping results in accumulated intracellular lipids and enhanced oxidative stress, inflammatory responses, and macrophage apoptosis. Thus, hepcidin is essential for ox-LDL–mediated phenotypic switching of iron-loaded macrophages leading to atherosclerotic plaque destabilization. Fpn1, ferroportin 1; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; MMP-2, matrix metalloproteinase-2; Ox-LDL, oxidized low-density lipoprotein; TNFα, tumor necrosis factor-α; SMC, smooth muscle cells. Caption text and figure reproduced with permission from Li et al.[115]

Increased ferritin: a surrogate marker or real risk factor?

Increased circulating concentrations of the iron storage protein, ferritin, are frequently observed in patients with CKD.[61, 116] However, like hepcidin, ferritin is also significantly upregulated in the acute phase response and particularly in the presence of low serum iron, transferrin and transferrin saturation, and is just as likely to reflect an inflammatory as an iron-replete state. In the general population, high serum ferritin concentrations are associated with an increased risk of myocardial infarction[117] and carotid plaques.[118] In patients with CKD, the associations between iron parameters and outcomes are confounded by multiple factors. One study reported that low serum iron is a predictor of poor outcome[119] even after adjustment for ferritin and the inflammatory marker C-reactive protein (CRP). In contrast, another observational study of

58,058 patients receiving HD showed an association between high ferritin (>800 ng/ml) and mortality, which was markedly attenuated following the correction for markers of malnutrition and inflammation.[89] Since correction for markers of inflammation markedly attenuated the risk associated with hyperferritinemia, prospective controlled studies are needed to assess if hyperferritinemia-associated CV risk merely represents a risk *marker* or is in fact a risk *factor*.

Can antioxidants blunt potential pro-oxidative effects of iron supplementation?

Although some studies have shown beneficial effects of a single dose of vitamin E[120] or 10 days of treatment of N-acetylcysteine[121] on surrogate markers of lipid peroxidation in patients on HD, it would be premature to recommend a single anti-oxidative therapy prior to iron supplementation due to the prodigious complexity of the system. Indeed, a study in 13 patients receiving HD showed that the combination of IV iron and vitamin C was actually associated with an *increased* production of reactive oxygen species.[122] It can be speculated that in the presence of poorly liganded iron, molecules that are normally antioxidants can actually act as pro-oxidants by reducing ferric iron to catalytically-active ferrous iron. A recent randomized controlled trial (RCT) in 353 HD patients examining the effects of six months of anti-oxidative therapy (tocopherols and α-lipoic acid) failed to influence biomarkers of inflammation and oxidative stress.[123] Thus, we currently do not know if increased oxidative stress in the uremic milieu responds to anti-oxidative treatment strategies. Since impairment of the Nrf2 system in uremia may render exogenous antioxidants alone less effective,[124] further basic science research regarding the mechanisms of increased protein, lipid and DNA oxidation in the complex pro-oxidative uremic milieu is needed.

IRON ADMINISTRATION AND RISK OF INFECTIONS

Experimental evidence

Iron is of central importance in host-pathogen interaction because of its key role in several biochemical and biological processes, including mitochondrial respiration and DNA synthesis, for virtually all cells and microorganisms.[125, 126] Accordingly, the proliferation and pathogenicity of many microorganisms such as bacteria, viruses, parasites, helminths and fungi, is dependent on the availability of iron.[127, 128] On the other hand, iron exerts subtle effects on host immune function by modulating immune cell proliferation and differentiation and by directly regulating cytokine formation and anti-microbial immune effector mechanisms. Data from recent reports suggest that oral iron has adverse effects on the gut microbiome composition, metabolism and virulence of intestinal pathogens.[129-131] Thus, imbalances of iron homeostasis can affect the risk for, and the outcome of, infections.[127, 132, 133]

Iron and immune function in dialysis patients

Immune cells have a varying demand for iron as iron availability is centrally involved in their differentiation and proliferation. For example, excess iron leads to modulation of both cytotoxic T-cell and T-helper (Th) cell proliferation, with the latter being characterized by an expansion of anti-inflammatory Th-2 cells. In patients on dialysis receiving IV iron, increased expression of the T-cell differentiating cytokine IL-2 along with an expansion of natural killer cells has been observed.[134] On the other hand, B-cell composition appears to be only slightly affected by perturbations in iron availability.[132, 135-137]

Monocytes and macrophages play key roles in the maintenance of iron homeostasis as both cell types take up and recycle senescent erythrocytes, a pre-requisite for balanced iron homeostasis because recycled erythrocyte iron accounts for approximately 90% of the daily needs of iron in the body.[125, 126] In addition, excess iron causes a shift in macrophage polarization from a pro-inflammatory M1 to an anti-inflammatory M2 type, which is characterized by increased expression of the heme scavenger receptor CD163 and heme degrading enzyme heme-oxygenase-1.[138] Although iron plays important roles in the innate host response by catalyzing the formation of toxic hydroxyl radicals via the Fenton reaction, excess iron inhibits anti-microbial effector pathways of macrophages.[128, 133] The immunosuppressive effect of iron overload is exerted via blockade of lipopolysaccharide (LPS) and interferon-gamma (IFNγ) inducible immune pathways, such as the formation of inducible nitric synthase (NOS2), tumor necrosis factor alpha (TNFα) or MHC class II expression, while macrophage deactivating cytokines such as interleukin-10 (IL10) are produced in excess.[139, 140] This appears to be of importance *in vivo* because therapeutic iron administration to patients receiving HD has been shown to result in a reduction of circulating TNFα.[79] In addition, IV iron has resulted in its

uptake and storage in circulating monocytes, which was more pronounced when serum concentrations of the master regulatory iron hormone, hepcidin, were increased.[80] Moreover, iron treatment caused a transient activation of the oxidative stress inducible transcription factor NF-κB along with stimulation of TNFα and IL-6 expression; however, iron loading over time as reflected by increased circulating ferritin concentrations resulted in an impaired immune response of circulating monocytes to *ex vivo* stimulation with LPS.[80] Additionally, different iron compounds exert divergent effects on monocyte differentiation and activity *in vivo*. A recent study indicated that iron sucrose had more prominent effects on monocyte differentiation than other clinically used iron compounds. Specifically, iron sucrose increased CD86 while simultaneously reducing CD16 and CX3CR1 expression on monocytes along with a reduction of their phagocytic capacity.[141] Along this line, iron loading has been associated with impaired function of neutrophil granulocytes, and parenteral administration of iron to patients on dialysis has been shown to decrease the anti-microbial killing capacity of these cells.[142]

Thus, it appears evident that iron loading may affect immune cell composition and activity over time, which can affect host responses to infection. However, it should be kept in mind that true iron deficiency may also adversely affect cell-mediated immune function due to its inhibitory effect on immune cell proliferation and differentiation, as has recently been confirmed by an observational study in anemic children.[143] Accordingly, we need to gain more insight into the effects of iron, and more specifically, of different iron preparations, on immune function in patients with non-dialysis-requiring and dialysis-dependent CKD, and how the effects of iron on immune function translate into risk for infections or other conditions where iron and inflammation play distinct roles such as intravascular oxidative stress and CVD.[144]

Biological plausibility

As iron is of central importance for pathogen proliferation, subtle changes in iron homeostasis occur during the course of infection. These systemic alterations of iron homeostasis are characterized by reduced circulating iron concentrations and hypoferremia, whereby iron is stored and restricted within macrophages. These alterations of iron trafficking lead to an iron-restricted erythropoiesis and materially contribute to the development of anemia of chronic disease, which in this context should be rather termed anemia of infection or anemia of inflammation.[139, 145] Several cytokines and acute phase proteins contribute to this diversion of iron transport, among which hepcidin appears to be of pivotal importance because its expression is induced by several cytokines and bacterial products such as LPS.[134] Hepcidin blocks macrophage iron transfer to the circulation by interacting with the iron export protein ferroportin, resulting in degradation of the latter and blockade of cellular iron release.[146] This iron restriction is considered as an attempt by the body to limit the availability of iron to microbes residing in the extracellular compartment, including the circulation.[127, 147, 148] Accordingly, several experimental models and observations from patients with genetic or transfusion-related iron overload have

demonstrated that excess iron in tissues or circulation can increase the risk for certain bacterial and fungal infections.[128, 149-151] Also in line with this observation there have been investigational trials carried out in areas with a high endemic burden of infections which have reported that dietary iron supplementation is associated with higher morbidity and mortality from infections.[152, 153] Accordingly, in areas with a high burden of infectious diseases, a certain degree of iron deficiency appears to be protective with respect to the incidence and severity of life threatening infections, such as *Plasmodium falciparum* malaria.[154] Similarly, higher risk of infection in kidney transplant recipients has been associated with higher serum ferritin concentrations.[155] However, it has yet to be proven whether higher serum ferritin in transplant recipients (as in patients receiving dialysis) is a surrogate for true iron loading or rather a combined reflection of iron levels and inflammation since ferritin is also an acute phase reactant[156, 157]

On the other hand, intracellular pathogens, such as Mycobacteria or *Salmonella*, may benefit from cytokine- and hepcidin-driven iron restriction in macrophages because they gain more access to intracellular iron which is necessary for their proliferation and pathogenicity.[148] Evidence from mouse models suggests that the host immune system has developed a specific strategy to restrict iron from such intracellular pathogens. Thus, the expression of ferroportin is increased by activation of stress and nitric oxide-inducible transcription factors, resulting in cellular iron egress and subsequently in limited availability of iron for intracellular pathogens along with a stimulation of innate immune functions.[158] Of note, *Salmonella sp.* are capable of counterbalancing this strategy by inducing hepcidin formation in the liver via estrogen-related receptor gamma activation; the increase in hepcidin leads to subsequent induction of macrophage iron retention and ensures sufficient access to iron for such intracellular microbes.[159] Interestingly, macrophages from mice or human patients with hemochromatosis share an improved control of infections with *Salmonella* and certain Mycobacteria because such individuals have lower serum hepcidin and lower macrophage iron concentrations,[160, 161] whereas patients with hemochromatosis are more vulnerable to infections with pathogens such as *Vibrio* or *Yersinia spp.*, which reside in hepatocytes, where iron concentrations are high.[162, 163] However, data are currently lacking from animal CKD models on the effects of iron perturbation on the course of infections and with pathogens that are of major relevance in CKD patients such as *Staphylococci*.[164] It should also be noted that susceptibility to infection in mouse models is significantly influenced by the genetic background and specific experimental conditions.

In addition, we need to gain more insight into the effects of iron perturbations not only on the incidence of infections, but also towards their potential for causing exacerbation of latent /chronic infection, such as tuberculosis, subacute bacterial endocarditis, or hepatitis C. Based on existing evidence from experimental models and observational studies, iron supplementation appears to have very distinct effects on pathogen proliferation depending on the compounds used, the underlying immune status, and most importantly the biology, pathogenicity and

localization of the specific microbe. This complex interaction between iron, immunity and infection is highlighted by the results of a recent prospective cohort study carried out in patients with tuberculosis and HIV infections. This study demonstrated that both iron deficiency and iron loading were associated with an adverse outcome of tuberculosis treatment.[165] Finding the right balance between iron deficiency and iron overload, identifying iron thresholds to ensure an optimal anti-microbial immune response and minimizing the risk of infection should be a major priority for future research in this area.

Clinical epidemiological evidence

Data from patients receiving HD

According to several published reviews, evidence amassed through 1999 was insufficient to determine if there was an association between iron therapy and a higher risk of infection in ESRD patients.[166-168] In January, 2014, Ishida and Johansen critically reviewed the association between iron and infection in patients receiving HD.[169] These authors identified studies which evaluated the association between serum ferritin concentrations (13 studies) and iron usage (24 studies) and the risk of infection.

Among the 13 studies that examined the risk of infection according to serum ferritin, nine reported an association and four did not. In general, studies showing associations between serum ferritin and infection compared higher (generally defined as >500 or 1000 ng/ml) and lower serum ferritin concentrations, and reported higher rates of bacterial infection and higher percentages of patients with infection in the higher serum ferritin groups. They also reported a 1.5 to 3.1-fold higher incidence of bacterial infection or infection-related mortality, which translates into an excess of 16 to 50 bacterial infections per 100 patient-years among patients with higher serum ferritin concentrations. Of the four studies showing no association, three were retrospective; of these, one was published in abstract form; another reported a non-significant difference in bacteremia-free tunneled catheter survival among 89 patients with serum ferritin concentrations >500 and ≤500 ng/ml, while another found a non-significant difference in the percentage of patients with infection, pneumonia or cellulitis/carbuncle among patients with serum ferritin >600 or < 600 ng/ml.

Out of the 13 studies mentioned above, only the EPIBACDIAL study prospectively examined risk factors for bacteremia among 985 patients receiving maintenance HD at 19 French dialysis units.[170] During a 6-month follow-up period, 51 episodes of bacteremia occurred, with an incidence of 0.93 episodes per 100 patient-months. In a multivariable Cox proportional hazards model, temporary catheters (particularly long-term indwelling catheters), history of two or more episodes of bacteremia, current immunosuppression, and lower Hb concentrations were independently associated with higher risk of bacteremia. However, there was no significant difference in serum ferritin concentrations among patients with (346 ± 502 ng/ml) and without

bacteremia (353 ± 434 ng/ml) [$p = 0.44$]. It is important to note that serum ferritin was an independent risk factor for bacterial infection in a previous study published by the same authors.[171] They attributed the discrepancy to a lower prevalence of iron overload (defined as serum ferritin concentrations >1000 ng/ml) in the later study (5% vs. 10%), which also reflected concomitant differences in ESA use (51.5% vs. 16.1).

In general, most of the 13 studies were retrospective and descriptive in terms of statistical analysis, had small sample sizes, and had problems with the definition of the predictor (as ferritin is an acute phase reactant) and the outcome variables (some only focused on sepsis/bacteremia or excluded certain types of bacterial infections). Furthermore, several studies were completed before the widespread use of ESAs and IV iron; since ESAs downregulate hepcidin expression, which is partially related to the formation of erythroferrone, and concomitantly exert immunomodulatory activities, their use might affect infection risk in general.[111, 172, 173] It is also unclear whether the risk of infection is different with iron overload caused by blood transfusions or IV iron.[169]

Among the 24 studies that evaluated iron usage and infection, the results were equivocal as 12 observational studies reported an association while 10 did not. Two RCTs also did not uncover an association though they were not primarily designed to assess the risk of infection. In the Dialysis Patients Response to IV Iron with Elevated Ferritin (DRIVE) study, 134 patients receiving maintenance HD with Hb concentrations ≤11 g/dl, serum ferritin concentrations between 500 and 1200 ng/ml, and transferrin saturation (TSAT) ≤ 25% were randomly assigned to receive 125 mg of IV ferric gluconate for eight consecutive HD sessions or no iron. Baseline erythropoietin dose was increased by 25% in both groups at randomization. After a 6-week follow-up, the incidence of any infection was not significantly different between the two groups (13 infections in 10 patients in the IV iron group and 12 infections in eight patients in the no-iron group).[174] In a 6-week observational extension of the study (DRIVE-II), the risk of infections was not significantly different between the two groups (4 infections in 4 patients in the IV iron group and 10 infections in 12 patients in the no-iron group); additionally, there was a lower risk of hospitalization from infection in the IV iron group than in the no-iron group.[175] In the second RCT, 42 patients receiving maintenance HD with Hb concentrations ≥9.5 g/dl, serum ferritin concentrations between 150 and 600 ng/ml, and TSAT between 19% and 30%, were randomly assigned to receive 4 to 6 loading doses of IV iron dextran, 100 mg each, over a 2-week period to achieve TSAT >30%, followed by 25-150 mg/week for 6 months to maintain TSAT 30-50% (n = 23), or to receive a fixed dose of 25-150 mg/week of IV iron dextran for 6 months to maintain TSAT 20-30% (n = 19).[11] In this study, one patient with multiple risk factors for infection died in the high TSAT group, and there was one admission for an infectious etiology in each group. Although this study and DRIVE were RCTs, they were not primarily designed to evaluate the risk of infection, were underpowered due to the small number of cases, and had a short follow-up (6 weeks in DRIVE and 6 months in the study by Besarab *et al*).

Of the remaining 12 studies showing an association between iron usage and infection, two large observational studies published in abstract form that used data from the United States Renal Data System (USRDS) reported a 14-45% higher risk of infection-related mortality with higher frequency and higher dose of IV iron.[169] Likewise, among 14,866 patients receiving maintenance HD at facilities managed by Dialysis Clinics, Inc (DCI) in the United States (US), higher mean IV iron dose per dialysis treatment (>34 mg) was independently associated with a higher risk of infection-related mortality at 6 months compared to a lower mean IV iron dose or no iron.[169]

Only two studies have examined the risk of infection with different IV iron formulations. In a single US center study, a comparison was made between two periods, one of only IV ferric gluconate use between April 2001 and January 2002 (period 1, P1) and another of only IV iron sucrose use between February and November 2002 (period 2, P2). A total of 63 patients received maintenance HD in both P1 and P2 (Group A) and 41 patients in either P1 or P2 (Group B). Adjusted relative risks for bacteremic episodes with iron sucrose vs. ferric gluconate were 2.92 (95% confidence interval [CI] 1.01-8.50) and 2.84 (95% CI 1.32-6.09) for Groups A and B, respectively. Adjusted relative risks for bacteremic episodes for IV iron doses >2000 mg vs. ≤2000 mg per year were 2.42 (1.03-5.63) and 1.54 (0.43-5.69) in Groups A and B, respectively, and thus, the association between IV iron dose and formulation and bacteremia was uncertain.[176] A second study enrolled 559 patients on maintenance HD from a single dialysis center in the US. Mean doses of IV iron sucrose and IV iron dextran between 2000 and 2006 were compared for patients who developed catheter-related sepsis (CRS) and patients who did not. Mean IV iron sucrose dose was significantly higher in patients with CRS (941 ± 1131 mg) than in patients without (553 ± 1131 mg) [$p = 0.001$], and also in patients who received IV iron dextran (483 ± 1255 mg vs. 191 ± 734 mg for CRS and non-CRS patients, respectively) [$p < 0.001$]. In addition, IV iron sucrose was independently associated with a lower sepsis-free survival, whereas the association was not significant for IV iron dextran.[177]

Only one study compared mortality with different dosing patterns of IV iron.[178] Based on data from 117,050 HD patients of a large US dialysis provider and the Medicare ESRD program, the authors estimated the effect of bolus vs. maintenance IV iron dosing during repeated 1-month exposure periods on risks of mortality and infection-related hospitalization during the subsequent 3 months. Among 776,203 iron exposure/follow-up periods, 24% of patients received high-dose iron (median monthly iron dose of 400 mg), 38% received low-dose iron (median monthly iron dose of 125 mg), and 38% received no iron. The percentage of patients receiving maintenance and bolus iron dosing was 49% and 13%, respectively, and the median monthly doses for each were 200 and 700 mg, respectively. In multivariable additive risk models, compared to maintenance dosing, bolus dosing was associated with a higher risk of infection-related hospitalization (risk difference, 25 additional events/1000 patient-years; 95% CI, 16-33), with the risk being highest among patients with a catheter or history of recent infection. An association between bolus dosing and infection-related mortality was also observed. In contrast,

maintenance or low-dose iron dosing was not associated with a higher risk of infection-related hospitalization or mortality outcomes when compared with no iron.

In general, the 24 studies had several limitations including: small sample sizes in some (<150) and short follow-up, issues regarding the definition of iron predictor and infection outcome variables, and statistics restricted to descriptive analyses in some studies, among others.[169]

More recent data

A multicenter study from Japan prospectively evaluated the association between serum ferritin concentrations measured quarterly for two years and IV iron usage with adverse outcomes and mortality among 1086 patients receiving maintenance HD. Using Cox proportional hazard regression models and including time-dependent covariates, the authors reported a significantly higher risk of infection with higher serum ferritin concentrations (consistently above 100 ng/ml) compared to lower ferritin concentrations (consistently below 100 ng/dl), and with high (≥50 mg/week) and even low (<50 mg/week) doses of IV iron compared with no IV iron. These authors also reported significantly higher risk of death among patients with high-amplitude ferritin fluctuations (serum ferritin concentrations consistently above 100 ng/ml or upward trend from below to above 100 ng/ml) compared with those with consistently low serum ferritin concentrations.[179] These results are interesting as the frequency and dose of IV iron and the mean serum ferritin concentrations among Japanese patients are far lower than in Western countries.[180]

In contrast to the Japanese study, other recent observational studies have reported mixed results regarding the association between IV iron and infection-related mortality or hospitalization. In the Dialysis Outcomes and Practice Patterns Study,[181] the outcomes of 32,435 patients on HD receiving IV iron in 12 countries from 2002-2011 were retrospectively analyzed. IV iron use was estimated as the average monthly dose received during the first four months of the study. Compared to patients receiving 100-199 mg/month (the most common dose range), those receiving an average of 300-399 mg/month or ≥400 mg/month had a higher mortality risk (HR of 1.13, 95% CI 1.00-1.27 for the group with 300-399 mg/month, and HR of 1.18, 95% CI 1.07-1.30 for the group receiving 400 mg/month or more of IV iron). However, this increased risk in all-cause mortality was not related to a specific disease, such as infections. Likewise, in an incident cohort of 9544 US dialysis patients, higher cumulative dose of IV iron was not associated with infection-related hospitalizations.[182] In contrast, a prospective, observational, single center study involving 235 incident dialysis patients followed for up to ten years found that patients who received IV iron had a significantly lower all-cause mortality ($p = 0.002$), including marginally lower sepsis-related mortality, although the latter did not reach statistical significance ($p = 0.06$).[183] No higher mortality was seen in patients with serum ferritin concentrations as high as 800 ng/ml as long as serum CRP concentrations were normal.

Two large observational studies by Feldman *et al.* deserve special comment because they highlight the difficulties of estimating the effect of IV iron on mortality and hospitalization, including hospitalization related to infection.[184, 185] In the first study of a non-concurrent cohort of 10,169 US patients on HD in 1994, data from 5,833 patients were entered in a multivariable Cox proportional hazards regression model. After extensive adjustment for 23 baseline demographic and comorbidity variables, administration of more than 10 vials of IV iron over 6 months (>1000 mg) vs. no vials was associated with a significantly higher risk of mortality (RR 1.11, 95% CI 1.00-1.24) and hospitalization (RR 1.12, 95% CI 1.01-1.25). In contrast, administration of 10 or fewer IV iron vials over 6 months (1-1000 mg) vs. no vials had no statistically significant association with survival (RR 0.93, 95% CI 0.84-1.02) or hospitalization (RR 0.92, 95% CI 0.83-1.03). In a second study by the same group of investigators, 32,566 US patients who received maintenance HD for at least one year during 1996 and 1997 were followed-up for all-cause mortality through mid-1998. Multivariable models that adjusted for 19 demographic, clinical and laboratory variables were fitted using either Cox proportional hazards regression or models that accounted for time-varying measures of iron administration, erythropoietin dosing, and several laboratory values, as well as other fixed- and time-varying measures of morbidity. In these analyses, administration of IV iron doses of more than 1000 mg over 6 months was associated with a significantly higher adjusted mortality hazard in the Cox model but not in marginal structural models with adjustment for time varying confounding, an approach that could mitigate indication bias.

Lastly, a meta-analysis that evaluated the safety and efficacy of IV iron therapy for functional iron deficiency reported no association of IV iron with higher risk of infection (RR 0.76, 95% CI 0.34-1.71). However, it only included two studies comprising 359 analyzable patients; as such, the conclusions are rather limited.[73] Two previous systematic reviews also evaluated the efficacy and safety of IV vs. oral iron in CKD 3-5D patients but did not report data on risk of infection.[186, 187] In contrast, a recent systematic review and meta-analysis of RCTs evaluating the safety and efficacy of IV iron therapy which included patients with either non-dialysis-requiring or dialysis-dependent CKD reported that, compared with either oral iron or no iron supplementation, IV iron was associated with a significantly higher risk of infection (RR 1.33, 95% CI 1.10-1.64).[188] However, since infection was not a predefined endpoint in many of the pooled studies, missing data could have introduced unmeasured bias in the analysis. Additionally, neither association between iron dose and risk of infection nor the higher risk of serious adverse events and mortality in patients receiving IV iron compared with oral or no iron were found. In view of these and other limitations, firm conclusions regarding the effect of IV iron on infection risk cannot be made from this meta-analysis.[188]

Data from peritoneal dialysis (PD) and non-dialysis CKD patients

Scant data are available regarding the effect of IV iron therapy and the risk of infection in PD or non-dialysis CKD patients.

In a retrospective analysis of 379 PD patients, 60 received two 500 mg IV iron injections, one week apart, of whom 32 received iron dextran, 23 iron saccharate, and five both formulations. Although not statistically significant, there were more peritonitis episodes during the six months after IV iron infusion, especially with iron dextran, compared to the peritonitis episodes during the six months before iron infusions (15 episodes vs. eight episodes respectively in six months). However, the study had only 36% power at the observed difference in peritonitis frequency.[189]

A recent randomized controlled trial by Agarwal et al.[190] comparing oral versus IV iron in non-dialysis CKD patients showed a higher rate of serious adverse events in the IV iron treatment group, with increased cardiovascular events and infections requiring hospital admission. However, this study examined a single center, with a single investigator adjudicating all serious adverse events and with only 99 subjects completing the trial. It is also of concern why the findings of Agarwal et al.[190] are so discrepant with those reported in the much larger FIND-CKD study,[97] a multicenter study conducted in 626 non-dialysis CKD patients worldwide and with considerably greater patient-years of follow-up. Despite patients being treated with much higher doses of intravenous ferric carboxymaltose (FCM) in FIND-CKD, no safety signals were evident, and indeed the incidences of infections (adverse events: 33.1% versus 34.0% versus 30.4%; serious adverse events (3.9% versus 3.3% versus 3.8%) and cardiac events (6. 5% versus 4.7% versus 4.5%) across all three groups (high-ferritin FCM, low-ferritin FCM, and oral iron, respectively) were identical.

In summary, the conclusions regarding the effect of IV iron on the risk of infection are as follows:

- The evidence derives mostly from observational studies conducted in HD patients.
    - The majority of studies that examined serum ferritin concentrations and infections found an association but they are prone to confounding due to ferritin being an acute phase protein. The results of studies evaluating iron usage were more equivocal.
    - There are only two RCTs in HD patients but they included a small number of patients with a short follow-up and were not specifically designed to assess the risk of infection with IV iron.
    - One observational prospective study from Japan suggests that there is higher risk of infection in patients with higher serum ferritin concentrations (>100 ng/ml) or who receive higher doses of IV iron (≥50 mg/week), whereas another observational prospective study found lower mortality, including sepsis-related

death, in incident dialysis patients receiving iron up to ferritin levels of 800 ng/ml when concomitant inflammation was absent. Two other large observational retrospective studies did not find an association between IV iron and mortality or hospitalization related to infections.

    - Several systematic reviews and meta-analyses are inconclusive.
- Data on PD and in patients with non-dialysis-requiring CKD patients are scarce.
- Despite inconclusive evidence concerning IV iron use and the risk of infections, current KDIGO guideline recommendations which call for balancing potential benefits vs. risks of IV iron therapy, as well as advising against IV iron use in patients with an active systemic infection, are still prudent.

HYPERSENSITIVITY

The safety of administration of IV iron compounds has been of concern to medical practitioners with the well-recognized risk of life-threatening adverse reactions to high molecular weight iron dextran and other older formulations. Although it is accepted that the dextran component of the formulation is likely to be the cause of these reactions, the general risk of parenteral iron administration needs to be clarified, particularly when many international jurisdictions promote home dialysis and medically unsupervised administration of parenteral iron. Furthermore, there are now newer formulations available that allow complete replacement doses in 15-60 minutes and novel methods of iron delivery have been developed (most notably through supplementation in the dialysate of patients undergoing HD, and also in iron-containing phosphate binders), some of which have now been approved in certain regions of the world. Thus, the conference attendees deemed it a high priority to assess the characteristics of reactions to IV iron and the pathogenesis of these reactions, as well as to provide advice on how these reactions should be managed both acutely and expectantly in different populations. Because of the rarity of these reactions, it was understood that the guidance provided here is largely driven by expert opinion.

Reactions to IV iron

Side effects to oral iron are common, occurring in up to 60% of patients.[191] These predominantly include an alteration in bowel habit (typically constipation) and nausea. Hence these side effects result in reduced adherence to oral iron intake. Anaphylaxis to oral iron salt supplementation has been reported but is extremely rare.[192]

IV iron was initially administered as iron oxide and was found to have an unacceptably high rate of toxic reactions.[193] Toxicity was largely thought to be attributed to labile iron, and subsequent iron preparations have been formulated with the iron salt encased in a carbohydrate shell, commonly a dextran polymer, sucrose, or gluconate. The resultant size of the complex determines the degradation kinetics, with iron dextran releasing iron more slowly than the lower molecular weight formulations. Hence, lower doses of iron sucrose and iron gluconate are recommended when given as a single infusion to minimize the risk of higher levels of labile iron and of potential reactions. With the exception of higher molecular weight iron dextran, the statistical differences in adverse reactions among different formulations cannot be quantified and are unlikely to be significant given the low incidence of reactions. However, a strong consensus is that higher-molecular-weight iron dextran should not be used, given that alternative formulations are now available with lower absolute risks of reactions. Although reactions to sodium ferric gluconate appear to be slightly more common than those seen with iron sucrose,[194] the absolute risk is low for both compounds.

In populations with non-dialysis-requiring and dialysis-dependent CKD, with or without concomitant ESA use, the advent of formulations available for more rapid infusion (e.g., lower molecular weight iron dextran, FCM, iron isomaltoside 1000 and ferumoxytol) could provide considerable benefit. These formulations may be viable alternatives to oral iron supplementation and despite their higher drug acquisition costs, may be cost-effective in certain healthcare settings.[195-198]

Characteristics of reactions to IV iron administration

Local skin reactions to extravasated iron can occur. Given the lack of clarity on the cause of systemic reactions to IV iron, we suggest a classification according to the severity of reaction, which can then be used to recommend the subsequent approach to both acute and longer term therapy (Table 1).

Table 1. **General classifications of drug hypersensitivity reactions**

Anaphylactic reactions
• Characterized by two or more organ systems involved (skin, gut, respiratory, CV) • Objective evidence of bronchoconstriction, stridor, hypotension, severe generalized urticaria, nausea, abdominal pain
Minor infusion reaction
• Often described as pressure in the chest or lumbar region, associated with flushing, with or without minor urticaria, but no hypotension or other organ involvement
Flare in pre-existing immune and/or inflammatory conditions, particularly rheumatoid arthritis
• Manifesting as arthralgia

It was agreed that it was generally not possible to predict those at risk for a hypersensitivity reaction. However, it was considered that the following patient characteristics may indicate a higher risk:

1. Prior reaction to any IV iron formulation
2. Moderate to severe asthma
3. Multiple pre-existing drug hypersensitivities or allergies[199]
4. Pre-existing immune mediated disease (e.g., autoimmune disorders)
5. Mast cell associated disorders

6. High transferrin saturation or low plasma transferrin levels, which may increase the likelihood of circulating labile iron during infusion[200, 201]

Infusion-specific risk factors such as use of higher doses and rapid rate of infusion[201] should also be considered when evaluating for any potential reactions. Whether generic formulations have a greater propensity for increased labile iron reactions is as yet unclear.

Anaphylactic (severe to life-threatening) reactions

Much of the data on this topic comes from pharmacovigilance studies which have severe limitations, including reporting bias, that should be considered when examining relative risks among different iron preparations. Nevertheless, one such study showed that higher-molecular-weight iron dextran had 3-4 times the rate of life-threatening adverse reactions at 11.3 per million patients compared with 3.3 per million patients for lower-molecular-weight iron dextran, and 0.9 and 0.6 per million population for ferric gluconate and iron sucrose, respectively.[202] Excluding high-molecular-weight-iron-dextran, which is no longer commercially available, anaphylactic reactions are extremely rare, with an incidence of <1:200,000. The FDA recently posted a regulatory update regarding severe hypersensitivity reactions with ferumoxytol, along with advice to slow down the rate of administration.[203]

To date, pharmaceutical filing and published trials have not demonstrated anaphylactic reactions with intra-dialytic administration of soluble ferric pyrophosphate,[204] oral ferric citrate,[42] or with another iron compound currently under development, heme iron polypeptide.[205] However, given the rarity of reactions with any form of iron administration, it cannot be concluded that oral or intra-dialytic administration of iron is without risk. Larger studies will be required, since the number of trial participants in current registrational studies is likely to be insufficient to show any signals of harm.

The consensus group considered that the pathogenesis of acute severe reactions is unknown and may be multifaceted. Clinical features such as urticaria, bronchospasm, and anaphylaxis are typical for the so-called immediate-type hypersensitivity reactions. These are often IgE-mediated, and more rarely caused by IgG-immune complexes, such as in the reactions to higher molecular weight dextrans. Iron itself is most likely not the antigen and carbohydrates are rarely involved in immune reactions. Potential pathomechanisms could include direct activation of basophils (e.g., by complement), an antibody-mediated reaction (IgG or IgE) to heretofore unknown allergenic determinant, or a "toxic" reaction. The possibility that labile iron may mediate acute severe reactions remains possible, but has not been proven.

So far there is no established and validated allergological work-up such as skin testing or *in vitro* tests available to predict or confirm hypersensitivity. Improved clinical documentation of hypersensitivity reactions to iron in the future should also include an allergological work-up to

identify possible, but as yet unproven, risk factors such as asthma, mastocytosis, concurrent use of drugs such as beta blockers and angiotensin-converting enzyme inhibitors, and the atopic status. Both iron and folate deficiency have been reported as risk factors for urticaria, but their role in contributing to reactions as a consequence of IV iron administration is unknown.[206]

Minor infusion reactions

Minor infusion reactions are not uncommon and may be characterized by symptoms such as flushing, mild chest discomfort, dizziness, light-headedness, nausea, or itching. In practice, asymptomatic hypotension is sometimes observed but this is considered a non-specific reaction unless iron is a known allergen for the patient from prior administration. Some patients may develop myalgia or arthralgia (the so-called 'Fishbane' reaction), which is usually self-limiting and does not require treatment with adrenaline or anti-histamines. These should generally not preclude the ongoing use of IV iron preparations. Again the causes of these reactions are not clear and may be multifactorial. Indeed, if minor infusion reactions occur when iron is being administered to patients on HD, it may be due to factors inherent in the dialysis procedure itself, such as a dialyser reaction. However, it may be that labile iron may contribute to the reactions.

Labile iron is redox active, exchangeable between ligands and is chelatable. The terms ‘free iron’ and ‘non-transferrin bound iron’ should not be used as they do not adequately define the labile iron pool. These mild infusion reactions may be diagnosed via their ability to resolve when the infusion is stopped or given at a slower rate.[207]

Generic iron formulations have been associated with higher reaction rates[208] and when measured, labile iron may be higher in these formulations. Given that low transferrin levels will result in reduced iron binding and therefore increase the propensity for increased labile iron, low transferrin levels are considered a theoretical risk for the development of minor infusion reactions. Extrapolating this concept would suggest that malnourished or nephrotic patients may be at increased risk of a reaction. However, there is no direct evidence of this and further study is required.

Management of hypersensitivity reactions to IV iron

Patients who have had a life-threatening reaction to IV iron should not receive further IV iron compounds. However, it was agreed that if patients experienced more minor features of hypersensitivity, then an alternative formulation could be tried at a later date with appropriate monitoring.[209]

A consensus algorithm for the management of reactions to IV iron is shown in Figure 3. Ring et al.[210] have summarized the optimal clinical treatment practice of severe anaphylaxis: this includes adrenaline as an essential anti-anaphylactic drug, which should be given by intramuscular injection 0.5 mg 1/1000 solution. This should be repeated after 5-10 minutes if needed. Additional supportive oxygen should be given at a high rate (>15 liters/min) by face

mask. Volume loading should be given using 1 liter of crystalloid solution in addition to an anti-histamine (H1 blocker) and corticosteroids to prevent a protracted or biphasic course of anaphylaxis (acknowledging that they offer little additional value during an acute episode).

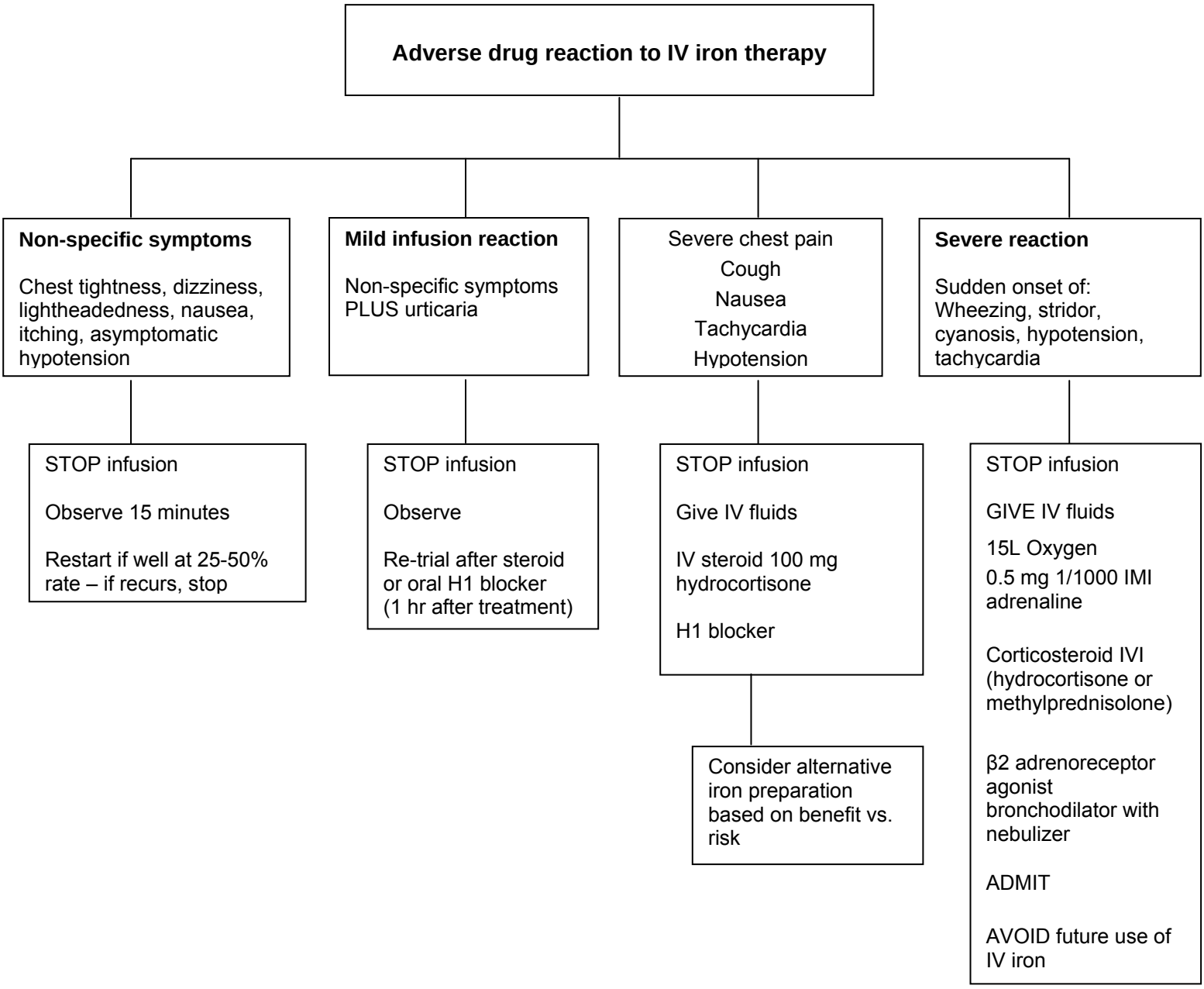

Figure 3. **Suggested management of reactions to IV iron.** IMI, intramuscular injection; IV, intravenous; IVI, intravenous infusion.

For non-specific reactions (e.g., symptoms such as mild chest discomfort, dizziness, light-headedness, nausea, itching, asymptomatic hypotension), stopping the infusion for at least 15 minutes and monitoring the response (i.e., pulse, blood pressure, respiratory rate and oxygen saturation) may be sufficient. If the patient improves, then the iron infusion can be resumed at 25-50% of the initial infusion rate with monitoring. For mild reactions, if treatment is re-started, IV H1 blockers and corticosteroids should be considered. Monitor after therapy for one hour. If the infusion is discontinued and the reaction subsides, then re-challenge with the same or a different iron preparation may be undertaken in an environment where monitoring is available. Consideration can be given to administering a much lower dose of the iron preparation or at a slower infusion rate to gain reassurance that this reaction is likely to be dose-related and possibly due to labile iron release.

It is not possible to reliably predict patients who will develop acute hypersensitivity with initial dosing of IV iron. However, as indicated above, it was agreed that patients with severe or uncontrolled asthma, or those with documented allergies to one or more substances, may be at a small but higher risk of a reaction to IV iron.

There is no evidence that pre-treatment with corticosteroids or antihistamines (H1 channel blockers) reduce the risk of severe reactions to IV iron. Paradoxically, IV antihistamines may be associated with unwanted side-effects, particularly drowsiness or flushing upon rapid infusion.[211] Hence no pretreatment with corticosteroids or antihistamines is recommended in patients identified as being at potential risk of a hypersensitivity reaction, but may be considered on an individual basis and, as always, clinical judgment should be applied. Desensitization protocols to limit hypersensitivity reactions are not established and therefore, not recommended.

Jurisdictional requirements regarding the use of IV iron vary, and clearly take precedence over the views of this consensus committee. It is recognized that in regions such as Australia where home dialysis is relatively common and patient-administration of IV iron supplements is routine, no excessive adverse event rate has been reported. In contrast, in 2013, the European Medicine Agency (EMA) made recommendations following reports of several hypersensitivity reactions in three pregnant women receiving low-molecular-weight-iron dextran compounds,[212] all of whom made a complete recovery. The recommendations were extrapolated to all patient groups receiving any IV iron compounds.

The workshop attendees agreed with the current position of the EMA that all IV iron preparations can rarely cause hypersensitivity reactions. It was agreed that data on the risk of hypersensitivity comes mainly from post-marketing spontaneous reports, and the total number of life-threatening reports is low. Although the data show a clear association of iron medications and hypersensitivity reactions, the data cannot be used to detect differences in the safety profiles of different formulations. The consensus group concurred that IV iron should not be

administered in the first trimester of pregnancy. It was also agreed that a test dose was not useful in any circumstance to predict the risk of hypersensitivity to IV iron.

Given the low likelihood of a reaction to IV iron, the conference attendees recommended the following:

- The first dose (either in a CKD or dialysis setting) should be administered in a clinical facility.
- Although total dose iron infusions have not been demonstrated to have significant risk,[213] IV iron doses of iron gluconate or iron sucrose should not exceed 125 or 200 mg/dialysis, respectively, due to the potential risk for iron not binding immediately to transferrin and resulting in a reaction due to labile iron.
- There is no physiological basis to recommend that patients should be observed for 30 minutes after an infusion of iron is completed since IV iron delivery should not be associated with a severe delayed reaction (as is observed with subcutaneous antigen presentation e.g., vaccination or allergen immune therapy). This was the singular point where the consensus group dissented from the FDA and EMA recommendations.

However, it should be reiterated that healthcare professionals are required to comply with jurisdictional recommendations and restrictions in their respective countries.

RESEARCH RECOMMENDATIONS

- The contribution of different factors to iron deficiency remains unclear and the mechanisms of reduced iron absorption, including the role of a low protein diet and the effects of concomitant drugs are still poorly understood and should be further clarified. A better understanding of the mechanisms and determinants of oral iron absorption could eventually lead to the identification of predictors of iron absorption that might help to stratify patients for trials of oral iron.

- Studies providing estimates of iron loss are generally limited and most account for procedure-related and lab test-related losses only, but not GI loss. More precise estimates of iron loss should therefore be performed in larger and unselected HD and non-HD patient CKD populations, with a particular focus on GI losses.

- While there has been a substantial focus on iron therapy, strategies to minimize blood loss appear to be a neglected area. Changes in practice, including altering rinsing procedures after dialysis, reducing blood sampling for lab tests, and reducing the use or duration of use of tunneled catheters for HD vascular access have the potential to reduce iron losses and thus the need for iron replacement.

- The development of a methodology to objectively determine body iron stores and tissue distribution in CKD and ESRD patients would be highly valuable.

- Studies should evaluate whether thresholds for increased risk of organ damage in patients with HFE-hereditary hemochromatosis (i.e., TSAT > 45%, ferritin >1000 µg/l) are applicable to patients with CKD and whether less strikingly abnormal values are also markers for harm.

- Further research is needed to determine if the administration of iron preparations to CKD patients produces adverse effects through different mechanisms and with different manifestations, and whether iron accumulation may potentially aggravate other comorbidities in CKD patients (e.g., viral hepatitis, non-alcoholic steatohepatitis).

- Studies should be conducted to determine whether treatment with iron has clinically relevant beneficial effects beyond stimulation of erythropoiesis in patients with CKD. This concept has been reported in patients with CHF,[214] as well as in patients with pulmonary arterial hypertension,[215] restless leg syndrome,[216, 217] and premenopausal women with low ferritin levels.[218, 219]

- Studies should determine if patient-centered outcomes (e.g., quality of life) provide an additional basis for iron therapy beyond the anticipated reduction in ESA requirements or improved Hb response.

- Given the recent trend towards more extensive use of IV iron to treat CKD-related anemia and the lack of conclusive evidence regarding long-term safety with IV iron therapy including infection,[220] there is an urgent need for RCTs to assess the relative safety and efficacy of IV iron in the management of CKD-related anemia, particularly in relation to hard clinical endpoints, as well as infection risk and other patient-related outcomes. Additional methodological aspects of RCT design to consider include: a) random allocation of patients to high-dose vs. low-dose IV iron, high vs. low serum ferritin target, bolus vs. maintenance dosing, and different IV iron formulations vs. placebo; b) use of cluster RCT (i.e., randomized to facility practice); c) use of rescue therapy for patients who develop iron deficiency to maintain the Hb level above 9 g/dl (10-12 g/dl); d) use of a fixed dose of ESA; e) inclusion of outcomes such as ESA dose, blood transfusions, infection, mortality, CV events (e.g., stroke and myocardial infarction), quality of life, and other patient-related outcomes.

- Observational studies should be conducted in kidney transplant recipients, patients with non-dialysis-requiring CKD and in patients receiving peritoneal dialysis, to determine infection and CV risks, and possible benefits with IV iron in these populations.

- Experimental studies using uremic animal models should be performed to test the effects of IV iron on active infection and the risk of developing new onset infections with pathogens most commonly encountered in the CKD population (e.g., *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, and Gram negative bacteria).

- A standardized questionnaire should be used to report *any* adverse reaction from an IV iron preparation. This was recommended by the EMA using an adapted version of Ring and Messner's classification of adverse drug reactions.[221] If implemented, this questionnaire could be used across other jurisdictions. This should help in the future to identify patients at risk for IV iron preparations that carry a higher risk of adverse drug reactions.

- Future research should ideally address the value of tryptase measurements in acute hypersensitivity reactions. Importantly, measurements should not be taken immediately after a reaction, but at least one hour after the onset of symptoms and supplemented by a baseline tryptase measurement a few days later. Additional measurement of complement factors C3a/C5a and C4 could provide information on the presence of immune-mediated reactions.

CONCLUSION

There is a plethora of laboratory and animal data to suggest that IV iron can exacerbate oxidative stress and potentiate infections. There are also observational studies that suggest an increased hazard ratio for all-cause, CV, and infection-related mortality, although there are other observational studies that do not show any increased risk. Data from RCTs are very sparse, particularly with regard to hard clinical endpoints. The amount of IV iron that can be safely administered is also not clear, and the traditional biomarkers of iron status such as serum ferritin and transferrin saturation are not particularly helpful in this regard. Manifestations of organ dysfunction as seen in HFE-hereditary hemochromatosis, a genetic condition of iron overload, is believed to be rare, but it is possible that patients receiving HD do not live long enough to develop this. Recent developments in MRI scanning have attempted to quantitate iron loading, but since they do not provide information on body iron distribution, its clinical relevance is as yet unclear. Hypersensitivity reactions may occur with all IV iron preparations, but are extremely rare now that the high-molecular-weight iron dextran compound is no longer available.

In summary, present available data do not allow any firm statement to be made on the potential dangers of high-dose iron administration and high ferritin levels and this conference has concluded that RCTs are urgently required to address the shortfall in the evidence base. One such trial, PIVOTAL,[222] is already well underway. The study is recruiting 2080 patients receiving maintenance HD across 55 sites in the UK who are being randomized to a high-dose vs. a low-dose IV iron regimen with a planned follow-up of between 2 and 4 years. Hard clinical endpoints such as death, myocardial infarction, stroke, heart failure, and infections are being assessed. In the meantime, nephrologists would do well to recognize broadly the benefits and the limitations of IV iron therapy, pending further robust scientific data.

DISCLOSURE

ICM declared having received consultancy fees from AMAG, Pharmacosmos, Takeda and Vifor; speaker honoraria from Vifor; and research support from Vifor Fresenius Medical Care Renal Pharma. AJB declared having received speaker honoraria from Vifor. K-UE declared having received consultancy fees from Amgen, Johnson & Johnson, Sandoz-Hexal, and Vifor and speaker honoraria from Amgen and Roche. GTO declared having received consultancy fees from Abbvie and Amgen and speaker honoraria from Abbott Nutrition, Abbvie, and Amgen. CAP declared having received consultancy fees from Amgen Australia, Astra Zeneca, Boehringer Ingelheim, Janssen Cilag, and Merck Sharp & Dohme; speaker honoraria from Astra Zeneca, Janssen Cilag, and Merck Sharpe & Dohme; and research support from Australian Research Council, Diabetes Australia, Hillcrest Foundation and JDRF. PS declared having received consultancy fees from Abbvie, Amgen, Keryx, Pfizer, and Vifor and speaker honoraria

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