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REVIEW

Iron metabolism in transplantation

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Summary

Recipient's iron status is an important determinant of clinical outcome in transplantation medicine. This review addresses iron metabolism in solid organ transplantation, where the role of iron as a mediator of ischemia–reperfusion injury, as an immune-modulatory element, and as a determinant of organ and graft function is discussed. Although iron chelators reduce ischemia–reperfusion injury in cell and animal models, these benefits have not yet been implemented into clinical practice. Iron deficiency and iron overload are associated with reduced immune activation, whose molecular mechanisms are reviewed in detail. Furthermore, iron overload and hyperferritinemia are associated with poor prognosis in end-stage organ failure in patients awaiting kidney, or liver transplantation. This negative prognostic impact of iron overload appears to persist after transplantation, which highlights the need for optimizing iron management before and after solid organ transplantation. In contrast, iron deficiency and anemia are also associated with poor prognosis in patients with end-stage heart failure. Intravenous iron supplementation should be managed carefully because parenterally induced iron overload could persist after successful transplantation. In conclusion, current evidence shows that iron overload and iron deficiency are important risk factors before and after solid organ transplantation. Iron status should therefore be actively managed in patients on the waiting list and after transplantation.

Introduction

Solid organ transplantation is a life-saving intervention in selected patients with end-stage kidney disease, liver cirrhosis, hepatocellular carcinoma, advanced lung disease, short bowel syndrome, and heart failure. The review will focus on kidney, liver and heart failure as for other organs including pancreas, very limited data on iron status and transplantation have been published. Despite major advances in surgical procedures and perioperative care, challenges remain in how to best select patients for transplantation, how to manage immunosuppression and how to preserve graft function preoperatively and long term. Each of these factors is critically affected by iron. The aims of this article are to (i) review the cross talk of iron metabolism and transplant immunology, (ii) to discuss how iron status affects prognosis in end-stage kidney, liver and heart disease to guide optimal patient management on the waitlist and (iii) to critically review the role of iron chelation in organ procurement and preservation.

Iron metabolism

Iron's unique ability to accept and release electrons renders this element indispensable for life, in particular energy metabolism and growth. Because of its high reactivity, iron uptake,—transport and—storage are tightly controlled [1]. The iron transport protein transferrin delivers the metal safely to cells requiring iron, mainly red blood cell progenitors. The bone marrow requires about 20 mg of iron per day for red blood cell production, most of which is provided from recycled red blood cells by macrophages. Excess iron is primarily stored in hepatocytes as ferritin [2]. Ferritin is also secreted into the circulation, where concentrations of ferritin in plasma are a surrogate parameter of body iron stores. However, interpretation of plasma ferritin

requires additional iron parameters, because hyperferritinemia is also increased in inflammatory conditions, where in contrast to iron overload, serum iron is typically low and transferrin saturation reduced. These changes are mediated by the iron hormone hepcidin, whose production is induced by the proinflammatory cytokines. On a molecular level, hepcidin inhibits iron release from macrophages by inactivating the only known cellular iron export pump ferroportin [3,4]. Taken together, iron is highly redox active, and immune activation is therefore associated with iron sequestration as a host defense mechanism (Fig. 1) [5].

Iron and preservation of graft function

Determinants of graft function also include organ size and function prior to procurement, donor age, cold and warm ischemia times, the redox status, and the cytokine profile. During ischemia, iron as the major redox active element is released from cells and has been implicated in the ischemia–reperfusion injury. Reduction of chelatable iron with deferoxamine during organ preservation reduces ischemia–reperfusion injury, which results in prolonged graft viability and function in different animal models [6–8]. Organ donation after cardiac death is associated with longer warm ischemia time and hence more severe ischemia–reperfusion injury, which can be significantly reduced by addition of iron chelator to the organ preservation solution [9]. Niu and co-workers recently reported that i.v. infusion of 20 mg/kg body weight of deferoxamine 30 min before induction of cardiac arrest significantly reduced AST and LDH levels and reduced the expression of proinflammatory cytokines from liver grafts in a non-heart-beating rat liver model [7]. Furthermore, cold ischemia could also be reduced as documented by improved renal blood flow, and reduced post-transplant creatinine concentration in a rat model of kidney transplantation [8]. Finally, when added to the cardioplegic solution, deferioxamine improved cardiac output and apoptosis in the myocardium [10].

Taken together, in organ procurement and preservation, iron chelation has a well-documented effect on reducing ischemia–reperfusion injury in animal models.

Iron and the allo-immune response

Iron and T-cell activation:

Solid organ transplantation causes allo-immune activation, whose intensity is co-determined by iron status. A three signal model has been proposed to understand allo-immune-mediated T-cell activation [11]. Each of these is not only a target for specific immunosuppressive drugs but also attenuated by iron (Fig. 2).

Signal 1 is mediated by interaction of the T-cell receptor with MHC class II molecules presenting allo-antigens expressed by dendritic cells and macrophages. The T-cell receptor is co-expressed with CD3 and CD71, which is the transferrin receptor that is required for iron acquisition from transferrin. Hence, the transferrin receptor is a T-cell activation marker, whose expression increases about 10-fold after T-lymphocyte activation with anti-

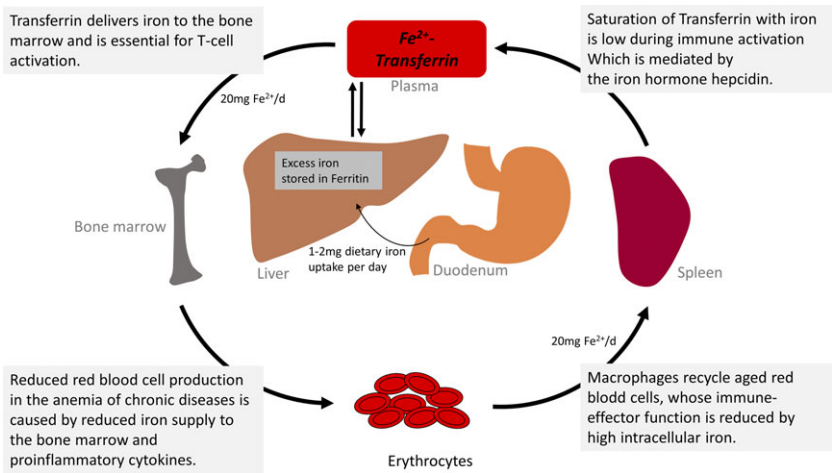

Figure 1 The iron cycle in relation to transplantation—each step of the iron cycle can be affected in transplant recipients. Each day 20 mg of iron delivered to the bone marrow for red blood cell production by the iron transport protein transferrin. Ferric iron bound to transferrin is also delivered by the circulation to activated T-cells. End-stage kidney, liver, and heart diseases and chronic inflammatory conditions after transplantation are frequently complicated by the anemia of chronic diseases. In this condition, proinflammatory cytokines inhibit red blood cell production, and high hepcidin levels reduce iron supply to the bone marrow by inhibiting iron release from macrophages. Macrophage iron accumulation is frequently present in chronic inflammatory conditions and associated with reduced immune effector function.

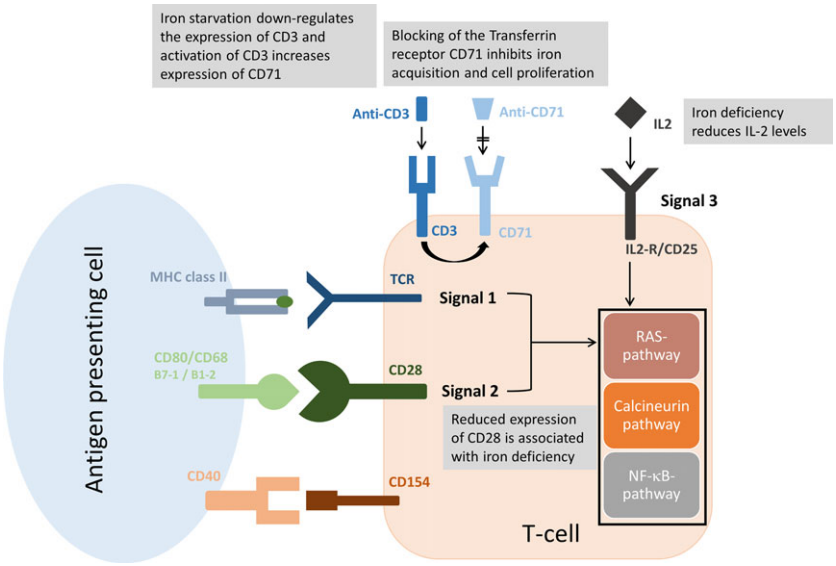

Figure 2 Allo-immune-mediated T-cell activation is regulated by iron: Allo-reactive T-cells bind transplant antigens through specific T-cell receptors when expressed by professional antigen-presenting cells as a complex with MHC class II molecules (Signal 1). Together with CD3 and CD71, the T-cell receptor is expressed as a complex that forms the 'immunological synapse', which activated intracellular signal transduction pathways (RAS, NF-κB, and calcineurin pathway) ultimately resulting in activation, proliferation, and differentiation of T-cells. Proliferation is directly dependent on iron. Iron starvation further reduces CD3 expression and IL-2 levels, which results in reduced immune activation during iron deficiency.

CD3 antibody [12], because iron is required for T-cell differentiation and proliferation [13]. Accordingly, blocking anti-CD71 antibodies have been shown to inhibit T-cell activation through iron starvation of T-lymphocytes [14]. This approach prolonged graft survival in a murine heterotopic, nonvascularized cardiac allograft model [15]. In addition, iron starvation by a chelator down-regulated the expression of CD3 *in vitro*, which is the target of the therapeutic monoclonal antibody OKT3 [16]. Taken together, iron is required for T-cell proliferation, and iron chelation was shown to inhibit T-cell activation.

The so-called co-stimulatory signal-signal 2-is also required for T-cell activation. This signal is mediated by the interaction of CD28 with B7 and CD154 with CD40. CD28 and CD154 are expressed on lymphocytes, whereas B7 and CD40 are expressed on antigen-presenting cells. Studies in mice have shown that iron deficiency is associated with reduced expression of CD28 [16], whereas the two activating ligands for CD28-CD80 (B7-1) and CD86 (B7-2)-are unaffected by iron [17].

The combination of signal 1 and signal 2 activates three downstream signal transduction pathways: (i) the calcineurin pathway, (ii) the RAS-mitogen-activated pathway, and (iii) the nuclear factor kappa B (NF-κB) pathway. Each of these signaling pathways is affected by iron. Calcineurin itself is an iron-dependent phosphatase, which requires the metal at the active site to dephosphorylate the transcription factor nuclear factor of activated T-cells (NFAT). Divalent iron at the active site renders the metal also sensitive to oxidative stress [18]. Furthermore, iron was shown to activate the RAS-mitogen-activated pathway and the NF-κB pathway in hepatic macrophages [19]. In turn, intracellular RAS signaling was recently found to control iron metabolism, by suppressing hepcidin expression [20]. This finding suggests that iron requirements for T-cell proliferation are maintained by suppressing hepatic hepcidin production. This effect is augmented by activation of hepcidin transcription in activated T-lymphocytes, which down-regulates iron release by inhibition of ferroportin through auto- and paracrine effects of locally secreted hepcidin [21].

Signal 3 of the T-cell activation pathway is mediated by IL-2 and IL-15, which deliver growth signals that are mediated through CD25/IL-2R. This signal can be therapeutically blocked by the monoclonal antibody basiliximab. Similar to the molecular action of basiliximab, iron deficiency was shown to be associated with reduced IL-2 levels in a cohort of patients with iron deficiency, which supports the notion that not only iron overload but also iron deficiency is associated with reduced immune activation [22]. The interaction of IL-2 with CD25 causes activation of the mTOR pathway. Hepcidin transcription was shown to be under control of mTOR in hepatocytes [20]. Accordingly, the mTOR inhibitor rapamycin was shown to induce hepatic hepcidin production in a cell culture model of human

hepatocytes. A recent study in heart transplant recipients has shown that treatment with mTOR inhibitors was associated with higher concentrations of circulating hepcidin, which could contribute to lower hemoglobin levels in these patients [23].

Iron, humoral and innate immune response

Although the allo-immune response is primarily T-cell mediated, humoral immune responses can be prohibitive for transplantation and are important for the formation of de-novo donor-specific antibodies. As an essential growth factor, iron is required for B-cell proliferation [24]. In contrast to T-cell activation and macrophage immune effector functions, which have been intensely studied in their relation to iron metabolism, few studies have focused on the functional interaction of B-cell activation, antibody production and iron metabolism [25,26].

In addition to specific cellular and humoral allo-immune responses, organ transplantation is associated with further immune activation of the innate immune system, which is not activated by specific allo-signals but through danger signals like metabolic or thermal stress associated with transplantation [27]. Furthermore, the innate immune system is key to the effector functions of the specific immune response [11]. The role of iron in controlling innate immune response is highlighted by the observation that binding activity of the complement factor C1q decreases in the presence of the iron compound hemin that is released as a result of tissue damage [28]. The impact of iron on the immune effector function of macrophages has been extensively studied, because macrophages are immune effector cells, which can also recycle and store iron. Macrophage iron storage is a stereotypic response during immune activation and in part responsible for the anemia of inflammation [29]. Iron-laden macrophages have been found to exhibit reduced immune effector functions [3,30].

In summary, iron and the immune system functionally interact in the context of transplantation in two compartments, which are iron in plasma and iron in macrophages. The T-cell-dependent allo-immune response requires iron from the plasma protein transferrin for activation and proliferation of T-cells. During acute phase responses, iron is withheld in macrophages which impairs their immune effector function and changes their cytokine expression profile. Taken together, iron deficiency mainly impairs the cellular immune response and iron overload primarily affects macrophage function. 'Functional iron deficiency' with low plasma iron and 'iron-trapping' in macrophages are prevalent among patients on the waiting list and believed to mediate impaired immune effector function. Specific changes in iron metabolism in end-stage kidney, liver, and heart failure and their impact on pre- and post-transplantation outcomes will be discussed in the following section.

Clinical relevance of iron metabolism in transplantation medicine

End-stage kidney, liver and heart disease are associated with typical alterations in iron metabolism. This section will review how these changes impact on patient wait-list survival, patient selection and post-transplant outcomes.

Iron and kidney transplantation

Hepatic iron accumulation and hyperferritinemia are prevalent among patients with end-stage renal disease (Fig. 3). According to current guidelines, target serum ferritin is 200–500 μg/l in hemodialysis patients to ensure supply of iron red cell production when erythropoiesis-stimulating agents (ESA) are given to treat renal anemia [31]. However, a recent study has shown that ferritin concentrations higher than 100 μg/l are associated with reduced survival in hemodialysis patients [32]. Before ESA were introduced in kidney transplant recipients, the prevalence of iron overload was as high as 28% [33]. Hepatic iron accumulation is also associated with an increased risk of liver disease, infection, and mortality [34]. Hepatic and splenic iron overload usually persists after successful renal transplantation because of the body's inability to excrete. Furthermore, recent studies have shown that high hepcidin concentration that are frequently present in dialysis patients only decrease in patients with adequate graft function [35]. The poor prognostic impact of hyperferritinemia (>1100 μg/l) also persists after transplantation [36]. As iron utilization by red

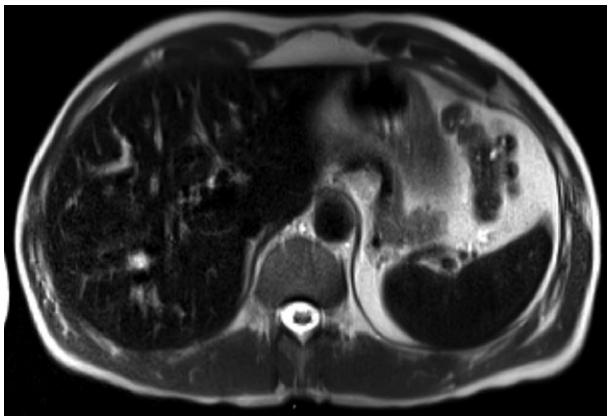

Figure 3 Hepatic and splenic iron accumulation in a kidney transplant recipient 3 years after transplantation. T2-weighted magnetic resonance imaging show marked reduction of signal intensity of the liver and spleen, which should normally appear isointense with the signal from the dorsal muscle.

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cell progenitors normalizes in transplant recipients with sufficient kidney function, iron could be safely removed by phlebotomies in such patients [37]. Although the effect of post-transplant iron reduction strategies on health-related outcomes has not yet been formally investigated in clinical studies, phlebotomy or iron chelation is expected to be effective in improving survival and reducing complications.

As iron deficiency is also common among hemodialysis patients, iron treatment has been tested to correct post-transplant anemia. A recent study showed no beneficial effect of oral iron on anemia but was associated with an increased rate of rejection [38,39]. This study epitomizes the risks associated with iron after transplantation and supports the concept that iron overload and hyperferritinemia are harmful after successful transplantation.

Iron and liver transplantation

The high prevalence of iron disorders in patients listed for liver transplantation and liver transplant recipients is because of the liver's function as central store for iron and because of its role in controlling plasma iron concentrations. The normal liver is the principal storage site for excess iron and the liver physiologically responds to proinflammatory signals (see above) and increased plasma transferrin saturation with hepcidin secretion. Reduced hepatocyte mass in patients with chronic liver diseases results in abnormally low hepcidin production and increased hepatocellular iron storage in remaining hepatocytes, regardless of the underlying etiology [40,41]. Accordingly, hepatic iron overload is present in over 30% of patients with liver cirrhosis [42]. Hyperferrtinemia (ferritin >200 μg/l) is associated with an unfavorable prognosis on the waitlist for liver transplantation, which is reflected by the fact that the prognostic performance of the model of end-stage liver disease (MELD) can be improved, when serum ferritin is taken into consideration [43,44]. This negative impact of high ferritin (>364 μg/l) on prognosis persists even after successful transplantation and affects 1-, 3-, and 5-year survival, which highlights the importance of iron beyond liver function [45].

The importance of iron metabolism for health is also highlighted by the observation that survival after liver transplantation for the iron storage disease hemochromatosis is significantly shorter than for other nonmalignant indications [46,47]. Reduced survival was attributed to an increased post-transplant rate of infections and to an increased risk for heart failure. Recent data suggest that the negative impact of increased hepatic iron is not restricted to hemochromatosis, where iron overload secondary to alcohol, hepatitis C or cryptogenic cirrhosis also appears to be a risk factor for reduced post-transplant survival [48].

The iron storage disease neonatal hemochromatosis is the most frequent nonidiopathic cause of liver failure during the first 90 days of life [49]. Although treatment with i.v. iron chelator and antioxidants have been effective in some cases, neonatal hemochromatosis is still an important indication for liver transplantation in newborns [50]. Although neonatal hemochromatosis can be potentially cured by liver transplantation [51], candidates for this therapy must be carefully selected because systemic disorders such as deoxyguanosine kinase deficiency can present as neonatal hemochromatosis, and neurological symptoms are very likely to progress despite successful transplantation [52–54]. A recent study suggests that neonatal hemochromatosis is caused by a gestational allo-immune disorder, where fetal liver failure is caused by the allo-immune response of the mother to the fetal liver, which causes intrauterine liver failure complicated by severe systemic iron overload in the newborn [55]. This group of patients should be considered candidates for neonatal liver transplantation because the gestational allo-immune liver disease can be cured by transplantation.

In the context of liver transplantation, iron is also involved in the regulation of graft tolerance as hepatic expressions of iron-related genes hepcidin, ferritin, and transferrin receptor 1 were found to be the most important predictors for the success of weaning from immunosuppression. This conclusion was drawn from a prospective study, where 75 liver transplant recipients who were stable at least 3 years after transplantation underwent liver biopsy. This biopsy was analyzed by whole genome transcriptional profiling before patients were weaned from immunosuppression. Of 72 patients, 33 had a stable liver function 1 year after weaning, whereas the remaining patients developed graft rejection. When transcriptomic profiles were analyzed for genes that could predict success of weaning from immunosuppression, low levels of transferrin receptor 1 (TRFC) & ferritin pseudogenes as well as high levels of hepcidin (HAMP) transcript were identified [56]. The gene signature of low transferrin receptor and high hepcidin is compatible with a high-iron phenotype of the hepatic graft, which—according to these results—favors tolerance.

Iron in heart and lung transplantation

Iron deficiency is a common cause for anemia in chronic heart failure and associated with an increased mortality when compared with nonanemic patients [57,58]. Likewise, anemia before cardiac transplantation is associated with a decreased 1-year survival on the waitlist, but this negative effect of pretransplant anemia did not persist after cardiac transplantation [59]. A clinical trial showed a positive effect of intravenous iron supplementation with ferric carboxy-

maltose on the functional status, symptoms and the quality of life in patients with heart failure and iron deficiency [60]. Assessment of iron status and treatment of iron deficiency could therefore improve patient-related outcomes for patients on the waitlist. For post heart transplantation patient care, avoiding iatrogenic iron overload by i.v. iron infusions will be key to optimize post-transplant outcome.

Few studies have reported on outcomes after heart transplantation for this indication. Caines *et al.* reviewed 16 patients with heart failure because of cardiac iron overload who received heart transplants. Actuarial 5- and 10-year survival rates were 81% and 41%, respectively, which is higher than the overall survival rates in heart transplant recipients [61,62]. Iron overload is a well-known cause of heart failure, which typically affects children with transfusional iron overload (Fig. 4) or juvenile hemochromatosis and can be prevented or cured by iron chelation therapy in some patients must be considered as differential diagnoses [63–65]. However, when cardiac iron overload is identified at the time of transplantation because of brown pigmentation of the heart, juvenile hemochromatosis or X-linked sideroblastic anemia must be considered as differential diagnoses [66–69]. In contrast to adult hemochromatosis, heart failure is highly prevalent in juvenile hemochromatosis, which is also associated with hepatic iron overload and organ damage [67,68,70]. Post-transplant care of patients with juvenile hemochromatosis will require combined treatment with chelation or phlebotomies to avoid re-accumulation of iron after successful heart transplantation [71–73].

In lung transplantation, pulmonary iron accumulation is a frequent complication. Baz *et al.* studied the iron concentration of bronchoalveolar lavage fluid and lung tissue in 10 lung allograft patients and compared them with a healthy control group. Tissue staining showed significantly increased levels of iron in alveolar macrophages and higher ferritin concentrations in macrophages although serum iron parameters did not show signs of iron overload [74]. These results were confirmed by more recent studies where hemosiderin scores in bronchoalveolar lavage samples were found to increase over time after transplantation [75,76]. The association of increasing iron concentration with an increasing number of acute rejections (grade ≥A2) suggests that acute rejection causes progressive iron accumulation [76].

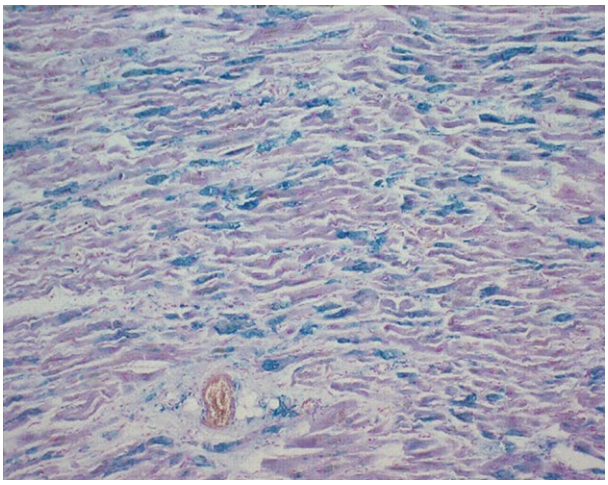

Figure 4 Perls' stained section of the heart muscle from a patient with cardiac failure secondary to repeated blood transfusions shows stainable iron as blue pigment in this micrograph.

Conclusions and perspective

Iron's inhibitory activity on T-cell proliferation and alloimmune activation is well understood on a molecular level. In the era of highly effective and selective immunosuppressants, this effect of iron is of little clinical relevance. Recent studies on the expression of iron-related proteins as predictors of success of immunosuppression withdrawal provide a new clinical perspective of the iron–immune interface [56]. However, these encouraging findings need to be confirmed in larger studies, before entering clinical practice. Likewise, iron chelation was shown to provide major benefits in organ preservation and graft survival, but iron chelation is not yet generally applied before organ procurement and during preservation.

Recent evidence shows that iron status is a critical determinant of patient-related outcomes in transplantation medicine [43]. This includes optimizing iron status on the waiting list because iron deficiency and iron overload are well established risk factors for death on the waiting list for liver, kidney, and possibly heart transplantation. Although the unfavorable impact of iron overload extends to post-transplant survival, no evidence-based recommendations on the definition and management of post-transplant hyperferrinemia can be given. Recent studies in hematopoetic stem cell transplanted patients with transfusional iron overload suggest that iron chelation improves survival [77]. Diagnosing iron overload in organ transplant recipients represents a significant challenge because immune activation and inflammation are also associated with hyperferritinemia, which is not necessarily an indicator of true iron overload in this patient group. Comprehensive assessment of iron status requires complete serum iron parameters including serum iron, transferrin, ferritin, and transferrin saturation, where patients with hyperferritinemia and elevated transferrin saturation are at particular risk. Direct assessment of iron storage requires a liver biopsy or magnetic resonance imagining with T2* relaxation times for noninvasive quantification of hepatic iron stores. Such

studies will be required to confidently assess the prognostic impact of iron on transplant-related outcomes.

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