Iron Overload in Patients Receiving Allogeneic Hematopoietic Stem Cell Transplantation

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Abstract: Iron overload has been associated to a variety of post-transplant complications, including infections, sinusoidal obstructive syndrome and it is conceivable that increased hepatic iron may mimic the clinical picture of GVHD or even may contribute to the worsening of hepatic GVHD.

Objectives and Methods: aim of present review was to summarize the current knowledge about diagnosis and treatment strategies of iron overload following HSCT.

Results: serum ferritin may be considered as surrogate marker of iron overload and is widely used as an indicator of body iron status; however other noninvasive diagnostic methods, namely SQUID and MRI, may provide more precise information on iron burden in specific organs, such as liver and heart. Since there are not physiological tools to remove efficiently the excess of iron, it is of particular importance to consider iron depleting therapy. Phlebotomy should be considered as the first line treatment of iron overload, while iron chelators, such as deferasirox, may represent an alternative option for patients with an inadequate hematological recovery.

Discussion: additional prospective studies are mandatory to investigate the relationship between iron overload and the outcome of patients receiving an allogeneic stem cell transplantation.

Keywords: Iron overload, Stem cell transplantation, GVHD, Iron chelators, Phlebotomy.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established therapy for a variety of malignant and non-malignant marrow disorders, however the risk of transplant-related complications represents a major drawback in the allogeneic HSCT setting. Age, disease and disease status may be considered as the main determinants of outcomes of patients with hematological malignancies undergoing HSCT. In addition to these factors, comorbidities as defined by a highly sensitive HCT-specific scoring system, have been shown to be strong independent patient-specific predictors of HSCT outcomes among patients with hematological disorders. Many different prognostic scoring systems have been developed, including a consistent number of variables potentially influencing the outcome of transplantation: among these, iron overload (IO) may be considered as a risk factor contributing to posttransplant toxicity. In fact, iron overload is a well-established adverse prognostic factor in patients receiving allogeneic hematopoietic stem cell transplantation (HSCT) for thalassemia [1, 2], but more recently it appears to play a similar role in patients with other hematological disorders [3-7]. In this respect, there are studies indicating that iron overload may be considered as a risk factor contributing to post-transplant liver toxicity, veno-occlusive disease (VOD), infection susceptibility and graft-versus-host disease (GVHD) and may negatively impact survival as well [8-10].

The purpose of present review is to summarize the knowledge on the mechanism of iron toxicity, the diagnosis and clinical impact of iron overload, and treatment options in HSCT.

IRON HOMEOSTASIS

Iron enters the body exclusively through the diet: approximately 1-2 mg of iron is absorbed from the diet every day. Iron is absorbed primarily in the proximal portion of the duodenum where is transported across the brush border: most dietary iron is in the ferric form (Fe^{3+}) and must be reduced to ferrous iron (Fe^{2+}) that enters the absorptive enterocytes through the divalent metal transporter 1 (DMT1). Once inside the intestinal epithelial cell, iron may be stored in ferritin or exported across the basolateral membrane of the enterocyte by ferroportin for delivery to transferrin and entry into the circulation; 65% of the iron is incorporated into red blood cell hemoglobin, while the rest is mainly stored in the liver, spleen, bone marrow, muscle and reticulo-endothelial macrophages. An average of 1-2 mg of iron is lost daily from the sloughing of skin and shedding of intestinal epithelium (1 mg/day), and in women, through menstruation (1 mg/day). Hence, there is not a mechanism to actively excrete iron from the body.

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Accordingly, iron homeostasis is basically regulated at the level of absorption by regulatory molecules such as hepcidin [11, 12]. Hepcidin is a 25-amino-acid peptide secreted by the liver considered to have a central role for regulation of iron metabolism: hepcidin binds to the iron transporter ferroportin, expressed on enterocytes and macrophages, which delivers iron from inside the cell to the circulation. In conditions of IO the hepatic expression of hepcidin is upregulated resulting in decreased iron absorption from the intestine and inhibition of release of storage iron by downregulating the expression of cellular iron export protein ferroportin [13, 14].

Hepcidin levels increase also in presence of inflammatory stimuli (IL-6), whereas its expression is decreased in iron-deficiency states [15, 16].

Due to the lack of an efficient iron export system, multiple transfusions lead to accumulation of iron: one blood unit contains approximately 200-250 mg of iron corresponding to the amount of iron we ingest with a normal diet over 6 months. As a result of transfusions, significant iron overload can occur after as few as 10-20 transfusions [12, 17].

IRON TOXICITY

The ability of iron to accept or donate an electron allows it to catalyze the formation of harmful reactive oxygen species (ROS) such as hydroxyl radicals that can damage cellular membranes, proteins and DNA. Under normal conditions, such catalysis is minimized by the sequestration of iron into complexes with transferrin. An increase in the body iron load may, however, saturate the available transferrin and lead to the presence of high levels of non-transferrin-bound iron (NTBI). NTBI is a very heterogeneous form of iron, the binding strength of which varies with the ligand. The component of NTBI with the weakest binding to plasma biomolecules is described as labile plasma iron (LPI), which is redox active and chelatable. As iron is only weakly bound to its ligand, LPI is able to permeate into organs and cause tissue damage [12, 17].

In patients with MDS, transfusion dependency represents the principal mechanism of IO, although other relevant factors such as ineffective erythropoiesis may play a crucial role [17, 18]. In allogeneic HSCT recipients, many different causes of elevated NTBI may be concomitantly present [17, 19]:

- Enhanced iron absorption due to anemia and underlying hematological malignancy (MDS)

- Dysregulation of intestinal iron absorption induced by alloreactive T cells [20].

- Red cell transfusions

- Inhibition of erythropoiesis as a result of preparative regimen

- Release of cellular iron as a result of tissue injury due to conditioning and destruction of bone marrow and tumor cells secondary to preparative regimen

In addition to iron toxicity due to the presence of ROS, clinical and animal data indicate that the presence of elevated available serum iron may predispose patients to bacterial and fungal infections. [8, 21-24] In fact, it is well known that iron is a key nutrient for bacteria and fungi, in particular mucorales: Rhizopus spp can accumulate up to a 40-fold greater amount of iron than can do Aspergillus fumigatus and Candida albicans [25-27].

Ferritin may also exert immunosuppressive effects on both T and B cells [28].

ASSESSMENT OF IRON OVERLOAD

Estimation of iron burden is primarily based on ferritin as a surrogate for IO [29], however many confounding factors particularly in HSCT recipients may result in potential IO overestimation. In fact, inflammation, infections, liver damage and GVHD may lead to elevated serum ferritin levels [5, 29, 30].

Since 90% of excess iron is deposited in the liver, liver biopsy with estimation of LIC (liver iron concentration) may provide and accurate measure of whole-body iron levels. Indeed liver biopsy with estimation of LIC is the validated reference method for evaluation of body iron stores, particularly in allogeneic HSCT recipients where alternative causes of hepatic dysfunction such as GVHD and infections should be excluded. Nevertheless, hematologic patients either during the pre-transplant period and after the transplant may be severely thrombocytopenic and at a high risk of bleeding during the procedure. Accordingly, noninvasive procedures for iron overload estimation may be considered as preferred tools in HSCT setting [17, 31, 32].

Superconducting quantum interference device (SQUID) has been used to quantify LIC after HSCT. The study of Busca *et al.* showed that approximately two thirds of the patients with hyperferritinemia had moderate to severe iron overload as estimated by

SQUID. Unfortunately, the very limited availability and the complexity of this methodology are an obstacle to the widespread clinical use of SQUID [33-35].

Quantitative MRI techniques, including T2 and R2 MRI have been shown to be a reliable method for noninvasive evaluation of LIC in both liver and heart (36), but more recently MRI imaging has also been adopted to define the impact of IO on outcomes following HSCT, because it can better reflect iron burden than does ferritin [37-39].

IMPACT OF IRON OVERLOAD ON SURVIVAL AND COMPLICATIONS AFTER HSCT

Estimation of IO in the allogeneic HSCT setting gained interest in recent years. Approximately 30% to 60% of allogeneic HSCT recipients may present a condition of IO either at the time of transplant or in the post-transplant period [35, 39, 40]. Indeed, several studies have investigated whether IO might have an impact on overall survival (OS) and transplant-related complications, namely bacterial and fungal infections, GVHD and hepatic dysfunction.

Survival and Non-Relapse Mortality

The harmful effect of IO on HSCT outcome was first demonstrated in patients with thalassemia. Lucarelli *et al* identified hepatomegaly, portal fibrosis, and the inadequacy of iron chelation therapy as independent risk factors for the outcome of thalassemic patients receiving HSCT [2]. According to these variables, patients were categorized in 3 prognostic classes associated to a different outcome: the 3 years probability of survival were 94%, 80% and 61% for class 1, class 2 and class 3, respectively [1].

The first evidence that IO may affect the outcome of patients with hematological malignancies has been reported by Altes *et al*. in 2002. The study showed in small cohort of patients (n=15 autologous and n=10 allogeneic HSCT) that high levels of ferritin (≥3000 ng/mL) and transferrin saturation ≥100% were associated with a reduced OS and increased transplant-related mortality (TRM) [4].

In 2007 Armand *et al*. published a retrospective study evaluating the prognostic impact of elevated pretransplantation serum ferritin in a large number of patients receiving an allogeneic HSCT [3]. The study including 590 adult patients who underwent myeloablative allogeneic HSCT, showed that elevated serum ferritin levels in AML and MDS patients were

associated with reduced overall and disease-free survival . The 5-year OS for patients with pre-transplantation serum ferritin in the first quartile (0–231 ng/mL) was 54%, in the second quartile (232–930 ng/mL) 50%, in the third quartile (931–2034 ng/mL) 37% and in the fourth quartile (>2034 ng/mL) 27% ($P<0.001$). The 5-year disease-free survival (DFS) rates, from lowest to highest quartile, were 43%, 44%, 34% and 27%, respectively ($P<0.001$). In MDS patients, the decreased survival was attributed to an increase in TRM ($P=0.002$). There was also a trend toward an increased risk of hepatic VOD in patients with high ferritin levels.

In line with these preliminary results, studies by Mahindra *et al*. have confirmed the important prognostic value of pre-transplant serum ferritin [41-43]. In patients receiving autologous transplantation, elevated pre-transplant serum ferritin levels were shown to adversely impact survival, relapse-free survival and relapse mortality [41]. Elevated ferritin was shown to be an independent adverse risk factor in patients conditioned with myeloablative regimens for allogeneic transplantation [42] Interestingly, pretransplantation ferritin >1910 ng/mL was associated with decreased incidence of both acute and chronic GVHD consistent with an immunosuppressive effect of iron burden as evidenced by the capacity of iron to reduce CD8+ T-cell counts [28] and influence innate and acquired immune responses [24, 27].

Similarly, in patients undergoing nonablative allogeneic transplantation, survival was also adversely affected by elevated ferritin [43-45].

One may argue that the transfusion load as mirrored by serum ferritin level may simply reflect the underlying disease stage [46].In this respect, results of a multicenter retrospective analysis of the influence of pre-HSCT serum ferritin (<1000 ng/mL [low] vs. ≥1000 ng/mL [high]) and disease status at transplant in 261 adults with AML, ALL and MDS have been reported [47]. Patients with high-risk disease were more likely to have serum ferritin ≥1000 ng/mL ($P=0.041$). Multivariate analysis showed that pre-HSCT serum ferritin and disease risk were independent significant prognostic factors for 5-year OS, DFS and non-relapse mortality rate (NRM). Patients with serum ferritin ≥1000 ng/mL had worse OS at 5 years compared with serum ferritin <1000 ng/mL (36% vs. 54%; $P=0.0001$). DFS (49% vs. 31%; $P=0.001$) and NRM (29% vs. 43%; $P=0.002$) were also worse at 5 years. Patients with serum ferritin ≥1000 ng/mL had a worse outcome than patients with serum ferritin <1000 ng/mL in both

standard-risk patients (OS: 54% vs. 64%; *P*=0.043; DFS: 46% vs. 57%; *P*=0.031) and high-risk patients (OS: 16% vs. 35%; *P*=0.001; DFS: 15% vs. 34%; *P*=0.001).

It has been demonstrated that in patients with MDS, transfusion dependency may be associated to a reduced survival after transplantation, and that the WPSS score, which includes transfusion as a prognostic variable, is able to stratify post-transplantation outcome in patients with MDS [48]. A large Italian retrospective study investigated the prognostic effect of transfusion history and iron overload on the post-transplantation outcome of 357 patients with MDS [49]. Transfusion-dependency was independently associated with reduced OS and increased NRM. Among transfusion-dependent patients receiving myeloablative allogeneic HSCT , pre-transplantation serum ferritin level had a significant effect on OS (*P*=0.01) and NRM (*P*=0.03) even after adjusting for transfusion burden and duration; as a consequence, it can be hypothesized that the negative effect of transfusion history on outcome might be determined at least in part by IO .

Indeed, a large number of studies have confirmed the predictive value of pretransplant serum ferritin levels on the main parameters of the clinical outcome (Table **1**).

By contrast, other studies did not support the evidence that pretransplant high ferritin levels might have a relevant impact on clinical outcomes.

Platzbecker *et al*. retrospectively analysed 172 patients with *de novo* MDS, demonstrating that iron overload resulted in higher NRM, while karyotype and marrow blast count appeared to be the overriding factors for survival [50].

The potential role of IO on posttransplant complications has been investigated in the autologous HSCT setting as well. A retrospective analysis of 224 patients who underwent HSCT assessed the clinical impact of pre-transplantation iron status on early transplant-related toxicity: 142 patients received autologous HSCT and 82 allogeneic HSCT for the treatment of AML (n=83), acute lymphoblastic leukemia (n=35), MDS (n=2), myeloma (n=45) and lymphoma (n=59). Median pre-transplant serum ferritin was 720 ng/mL (range 20–9255). Overall, 143 patients had serum ferritin levels <800 ng/mL (low serum ferritin group) and 81 patients had serum ferritin levels ≥800 ng/mL (high serum ferritin group). Allogeneic HSCT recipients with low pre-transplant serum ferritin had significantly lower

day 100 mortality than patients with high pre-transplant serum ferritin (p=0.003), while there was a trend toward a reduced TRM among autologous HSC recipients with low ferritin levels (p=0.006) [51].

Trottier *et al*. defined the impact of IO on transplant outcomes of 88 patients, using liver magnetic resonance imaging (R2-MRI) : 60 out of the 88 patients were defined as having IO , based on LIC > 1.8 mg/g. Median ferritin was 290 and 1732 in the no-iron overload and IO groups respectively. Pre-transplant ferritin levels only moderately correlated with LIC, but more importantly they did not observe an association between pre-transplant IO defined by R2-MRI measured LIC and OS , NRM, relapse rate, GVHD, bacterial, viral or fungal infections. [52] Similar results have been reported by Armand *et al*. in a cohort of 45 patients with acute leukaemia and MDS who received an allogeneic HSCT: although serum ferritin appeared to have prognostic significance, pre-HSCT IO assessed by LIC > 5 mg/gdw was not associated with increased mortality, relapse or GVHD [37]. A meta-analysis of 4 prospective studies evaluating the use of MRI to quantify pretransplant LIC failed to demonstrate a significant association between an elevated LIC (> 5 mg/gdw or > 7 mg/gdw) and OS , while a serum ferritin >1000 ng/ml resulted to be a significant prognostic factor (HR 1.7, p=0.36). A significant association between an elevated LIC (> 7 mg/gdw) and NRM , was demonstrated only in a subgroup analysis of patients who received a reduced intensity HSCT (HR 2.2, p=0.26) [53].

By contrast, Wermke *et al*. found that MRI-based LIC was an independent negative prognostic factor for NRM in 88 AML and MDS patients undergoing allogeneic HSCT: LIC 125 mol/g was a significant risk factor for NRM in uni-and multivariate analysis, and was associated with a decreased OS whereas serum ferritin and transfusion burden were not [38].

POSTTRANSPLANT COMPLICATIONS

Mucositis

Two studies addressed the issue of a potential impact of IO on the occurrence of severe mucositis. In a group of 50 patients receiving autologous HSCT, ferritin ≥1,500 µg/l was a predictor for severe mucositis either in univariate (50 vs 17%, P=0.05) and multivariate analysis (RR 2.3, P=0.028, CI 95% 1.3 to 79) [54].

In a retrospective analysis of 224 patients with haematological malignancies who underwent HSCT (n=142 autologous; 82 allogeneic HSCT), 143 patients

Table 1:   Studies Evaluating the Impact of Pretransplantation Serum Ferritin Levels on Clinical Outcomes

Ref.	Study	No. Patients - Diagnosis	HSCT	Cut-off for Pre-HSCT Serum Ferritin	Prognostic Impact			
					OS	DFS	NRM	Other Variables
Altes A BMT 2002 [4]	prospective	25 patients. Acute leukemia, CML, lymphoma	Autologous (15) Allogeneic (10)	≥ 3000 ng/mL	reduced	-	increased	-
Armand P Blood 2007[3]	retrospective	590 patients. Hematologic malignancies	Allogeneic (myeloablative)	≥ 2515 ng/mL	reduced	Reduced (AML, ALL, MDS)	Increased (MDS)	Increased risk of VOD
Altes A Ann Hematol 2007[54]	prospective	81 patients. Hematologic malignancies	Autologous (50) Allogeneic (31)	≥ 1500 ng/mL	-	-	-	Higher rate of severe mucositis, bacteremia, days with fever[2]
Mahindra A BBMT 2008 [41]	prospective	315 patients. HD and NHL	autologous	≥ 685 ng/mL	reduced	-	No effect	Higher rate of relapse
Mahindra A BJH 2009 [42]	retrospective	222 patients. AML, ALL, CML, MDS, NHL	Allogeneic (myeloablative)	≥1910 ng/mL	reduced	-	increased	Reduced rate of cGVHD
Mahindra A BMT 2009 [43]	retrospective	64 patients. Hematologic malignancies	Allogeneic (nonablative)	≥ 1615 ng/mL	reduced	-	-	-
Tachibana T Lek Lymph 2012 [47]	prospective	261 patients. AML, ALL, MDS	allogeneic	≥ 1000 ng/mL	reduced	reduced	increased	-
Pullarkat V BMT 2008 [9]	prospective	190 patients. Hematologic malignancies	allogeneic	≥ 1000 ng/mL	reduced	-	increased	Increased risk of aGVHD and BSI
Platzbecker U BBMT 2008 [50]	retrospective	172 patients. MDS	allogeneic	≥ 1000 ng/mL	reduced[1]		No effect	Increased risk of aGVHD; higher HCT-CI
Kataoka K BBMT 2009 [44]	retrospective	264 patients. Hematologic malignancies	allogeneic	≥ 599 ng/mL	reduced	-	increased	-
Kim YR A. Hemat 2009 [81]	retrospective	38 patients.Myeloid malignancies	Allogeneic (RIST)	>1578 ng/mL	reduced	reduced	No effect	-
Alessandrino Haematol. 2010 [49]	retrospective	357 patients. MDS	Allogeneic	≥ 1000 ng/mL	reduced	-	increased	-
Sucak GT Transpl Proc 2010 [82]	retrospective	250 patients. Hematologic malignancies	Allogeneic (148) Autologous (102)	≥ 500 ng/mL	reduced	-	Increased[3]	Higher rate of fungal infections,SOS, severe mucositis[4] pneumonia[4]
Tachibana T BMT 2011 [83]	prospective	119 patients. AML, MDS	allogeneic	≥ 1000 ng/mL	reduced	reduced	No effect	-
Armand P Am J Hem. 2012 [37]	prospective	45 patients. ALL, AML, MDS	Allogeneic (myeloablative)	≥ 1000 ng/mL	reduced	-	No effect	-

Abbreviations : VOD, veno-occlusive disease ; cGVHD, chronic GVHD ; aGVHD, acute GVHD ; BSI, blood stream infections; RIST, reduced intensity stem cell transplantation ; SOS, sinusoidal obstruction syndrome ;

1 lower 3-year OS ; no effect in multivariate analysis

2 the predictive value of pre-SCT ferritin levels was restricted to AUTO-transplanted patients in both univariate and multivariate analysis

3 Pre-HSCT ferritin concentration correlated with days 30 and 100 and with overall mortality ($P=.004$, $P=.001$, and $P= .002$, respectively) in allogeneic HSCT recipients

4 the predictive value of pre-SCT ferritin levels was restricted to AUTO-transplanted patients in logistic regression analysis

had serum ferritin levels <800 ng/mL (low serum ferritin group) and 81 patients had serum ferritin levels ≥800 ng/mL (high serum ferritin group): patients with low pre-transplant serum ferritin had significantly lower incidence of grade 3 and 4 mucositis than patients with high pre-transplant serum ferritin either after autologous and allogeneic HSCT (p=0.05 and p=0.03 respectively [51].

Infections

Several studies evaluated the relationship between infections and IO. Data from a retrospective review of bloodstream infections (BSI) in 114 consecutive patients with AML and MDS who received an allogeneic HSCT between January 2000 and December 2008

showed that BSI occurred in 36 of the 114 (32%) patients and the median time onset of the first BSI was day 28 (range day 0–95) after transplantation [8]. Interestingly, there was a significant difference in the incidence of BSI between the low ferritin group (<1000 ng/mL: BSI incidence 21%) and the high ferritin group (≥1000 ng/mL; BSI incidence 42%) (*P*=0.02).

Kanda *et al*. retrospectively analyzed the data of 112 consecutive adult patients with hematological malignancies who received an allogeneic HSCT without antibacterial prophylaxis. Overall, the cumulative incidence of bacterial infection at 30 days after transplantation was 16%. High serum ferritin (>700 ng/mL) and high C-reactive protein (>0.3 mg/dL,) levels were significantly associated with the development of bacterial infection in a multivariate analysis. In addition, septic shock and sepsis with organ failure were exclusively observed in patients who had high ferritin and/or high CRP levels [21].

Pullarkat *et al*. showed in a group of 190 patients with haematological malignancies that there was a significant difference between the high- (≥1000 ng/mL) and low-ferritin groups (< 1000 ng/mL) for the incidence of BSI (44% vs 60%, P=0.042). In multivariate analysis, the odds for developing BSI were higher in the high-ferritin category (odds ratio=1.99, Wald test P=0.032) [9].

In a prospective study, a ferritin level >1,500 ng/mL was associated with the presence of BSI and the number of days with fever following autologous HSCT, in both univariate and multivariate analysis, while in allogeneic HSCT recipients this variable did not reach statistical significance [54]. Virtanen *et al*. evaluated whether pretransplant IO measured by MRI was predictive of severe infections in 67 allogeneic HSCT recipients: hepatic IO (defined as hepatic iron concentration> 36 μmol/g liver dry weight) was present in 78% of the patients while cardiac R2* was normal in all patients. Hepatic IO was significantly associated with severe infections in the early posttransplant period and significantly reduced the risk of acute and chronic GVHD [55].

A retrospective study analyzed the significance of serum hepcidin levels as a predictor of infectious complications after allogeneic HSCT [56]. The cumulative incidence of documented bacterial infections at day 100 was evaluated according to pretransplant hepcidin levels: the incidence of bacterial infections, was significantly higher in the high-hepcidin group (≥50

ng/mL) than in the low-hepcidin group (<50 ng/mL) (65% vs 11%; P<0.001).

Several studies have evaluated the relationship between pretransplantation ferritin levels and the development of invasive fungal infections (IFI) [26, 57]

Sivgin *et al*. evaluated the pretransplantation serum ferritin levels in 73 patients with pneumonia: the median ferritin levels were 1705 ng/ml (41–7198) in the group of patients with invasive fungal pneumonia and 845 ng/ml (18–7099) in the non-fungal pneumonia group, respectively (p = 0.001). A threshold of 1550 ng/ml for serum ferritin indicated an increased risk of fungal pneumonia [22].

Similarly, Ozyilmaz *et al*. found in multivariate analysis that increased ferritin levels (>1000 ng/ml; OR: 3.42, 95% CI 1.03–11.42, P=0.045) and the development of sinusoidal obstruction syndrome (OR: 5.09, 95% CI 1.53–16.90, P=0.008) were significant risk factors for fungal pneumonia [25].

According to these findings the presence of IO has been now considered as a risk factor for the development of IFI in patients undergoing allogeneic HSCT [58].

Graft-Versus-Host Disease

GVHD is a major hindrance to the success of HSCT, contributing substantially to morbidity and transplant-associated mortality. . Acute GVHD usually develops at the time of engraftment and the incidence ranges between 20% and 70%, depending on the extent of histocompatibility mismatches, the age of the recipient and the intensity of preparative regimens.

GVHD is caused by donor T lymphocytes reactive to histocompatibility antigens of the host. Significant experimental data suggest that dysregulated cytokine production occurs after allogeneic HSCT as a cascade of events involving sequential T cell and monocyte activation and is responsible for many of the manifestations of GVHD. During the first phase, the release of proinflammatory cytokines (IL-1, IL-8, TNFα) may determine the initial histopathological damage in GVHD target organs. This initial cytokine release is further amplified in the second phase via the activation of donor T cells reacting to host tissue antigens, while the final phase involves further host tissue damage at the level of both effector cells and release of predominating Th1 type cytokines (IL-2, IFN- , TNF-) [59]. Based on these observations, it is possible to

speculate that host tissue damage exacerbated by iron burden may have detrimental effects for the development of GVHD.

The effect of elevated pretransplant ferritin levels (defined as ferritin ≥1000 ng /ml) on acute GVHD was assessed in 190 patients with hematological malignancies who received an allogeneic HSCT, showing that the high-ferritin levels increased the odds of GVHD and death by 3.11-fold - [9]. Another study showed that grade III-IV acute GVHD was significantly higher in patients with ferritin values > 2500 mg/L as compared to patients with lower ferritin levels (46 % vs 18 %; p=.031) [50]. Conversely, Mahindra *et al*. found that patients with pretransplant ferritin levels > 1910 ng/mL had a significantly lower incidence of limited and extensive chronic GVHD (26% vs 47%, p= 0.019) and a lower incidence of acute GVHD (48% vs 69%, p=0.10). [42] Similarly, high ferritin levels greater than 400 ng/mL were associated with a 50% reduced risk of chronic GVHD [60], supporting the evidence for the suppressive effect of iron burden on adaptive immune responses [28, 61].

Overall, these conflicting results strengthen the need for further prospective studies to address the association of IO and the occurrence of GVHD.

Hepatic Dysfunction and Veno-Occlusive Disease (VOD)

Since liver represents one of the target organs where iron may accumulate preferentially, we can expect that hepatic injuries following transplantation, might be more consistent in those patients with iron overload.

Liver dysfunction is a very common complication of allogeneic HSCT, occurring in 57%-80% of patients: GVHD, drug toxicity, VOD, and infections are the most common factors potentially responsible of liver dysfunction in allogeneic HSCT recipients. [62]. VOD usually occurs in the first 3-4 weeks after HSCT as a result of endothelial and hepatocyte damage caused by the chemo-radiotherapy of the conditioning regimen, however more recently, this form of liver disease has been renamed as sinusoidal obstruction syndrome (SOS) because the sinusoidal cells are the primary target of the toxic injury. [63]. IO has been identified as a potential risk factor for the development of SOS [64-65]: Armand *et al*. found that pretransplantation ferritin in the top quartile (≥ 2515 ng/nL) was associated with a borderline significant increase in the risk of VOD (*P=0*.054) [3]. Busca *et al*. found that 54% of the patients

with a post-HSCT ferritin level <1000 ng/mL had at least 1 abnormal liver function tests value, compared with 84% of the patients with post-HSCT hyperferritinemia (P=0.001), and multivariate analysis confirmed a significant correlation between hyperferritinemia and liver dysfunction. [35].

EVALUATION OF IRON OVERLOAD IN THE POST-TRANSPLANT PERIOD

In general, data regarding hyperferritinemia and IO after HSCT are scarce.

Majhail *et al*. evaluated the prevalence of IO in 56 recipients of allogeneic HSCT who survived for a median of 28 months from transplant: serum ferritin >1000 mcg/ml was present in 19 patients and 18 of these had IO as determined by R2 MRI (defined as a LIC>1.8 mg/g), resulting in an overall prevalence of 32%. [66].

Busca *et al*. have evaluated posttransplant IO by means of serum ferritin and SQUID in a cohort of 102 adult patients who received an allogeneic HSCT. IO assessment was performed at a median time of 578 days after HSCT, and 44% of the patients has serum ferritin level > 1000 ng/ml. A significant correlation was found between a ferritin level >1000 ng/mL and the presence of at least one abnormal liver function test, and the rate of proven/probable invasive fungal disease was significantly higher in patients with hyperferritinemia (13% vs 0%; P5.006) [35].

Meyer and colleagues analysed iron parameters in 290 patients who received myeloablative HSCT, showing that ferritin levels peaked during the first 3 months posttransplant and then tended to decrease to below pre-HSCT levels in long-term survivors: a serum ferritin level >1000 ng/ml was found in 62% of the patients before the transplant and in 64%, 51%, 38% and 12% of the patients at 6, 12, 24 and 60 months post-HSCT, respectively. More importantly, elevated pre-HSCT ferritin levels were associated with increased NRM and reduced OS, even in 6-, 12- and 24-month landmark analyses. The adverse effect of hyperferritinemia appeared to depend on both an increased risk of relapse and an increased risk of NRM. Taken as whole, the results of the study of Meyer *et al* suggest that interventions to reduce excessive body iron might be beneficial both before and after the transplant. [67].

Grobekatthofer *et al*. have investigated whether elevated post-HSCT serum ferritin might correlate to acute or chronic GVHD in 131 pediatric patients

receiving allogeneic HSCT. Although they reported a low incidence of acute GVHD grade II-IV (10%) and chronic GVHD (25%), the study showed that post-HSCT hyperferritinemia was significantly associated with decreased survival and multi-organ failure, while did not demonstrate any impact on both acute and chronic GVHD. [68].

THERAPEUTIC MANAGEMENT OF IRON OVER-LOAD AFTER HSCT

Given the potential detrimental effect of IO on the outcome of haematological patients undergoing allogeneic HSCT, there is a growing body of evidence supporting the removal of iron excess, although defined criteria for when and how to treat iron burden have not been established.

Guidelines Recommendations

The majority of the guidelines published so far have evaluated the management of IO in patients with MDS (69-72). All the Authors agree that MDS patients who are candidate to allogeneic HSCT with ferritin > 1000 ng/ml or transfusion requirement of 2 units per months for over 1 year should receive iron chelation before the transplant.

Indeed, two guidelines only have been developed to optimize the management of IO in patients receiving allogeneic HSCT.

A consensus promoted by GITMO provided recommendations to support the appropriate choice for IO assessment and treatment in patients with MDS receiving an allogeneic HSCT [73]. The guidelines recommend that, MDS patients candidate to allogeneic HSCT should be evaluated for IO considering transfusion history, previous iron chelation, serum iron level, total iron binding capacity, serum ferritin, and percentage of transferring saturation. In patients with clinical signs of liver and/or heart damage, MRI T2 for cardiac study and MRI R2 for liver iron study should be performed. Liver biopsy is not routinely recommended in these patients. The Expert Panel agrees that all MDS patients who are transfusion dependent and are potential candidate to allogeneic HSCT should receive iron chelation treatment to prevent IO. Nevertheless, it is worthwhile recalling that the achievement of a reduction in IO should not cause a delay in transplantation.

The EBMT Joint recommendations about screening and preventive practices for long term survivors after hematopoietic cell transplantation [74] recommend that liver function tests must be evaluated every 3-6 months for the first year after the transplant, and then at least yearly. Serum ferritin should be measured one year post transplant, however since ferritin value may be confounding as ferritin is an acute phase reactant, when its value is abnormal, confirmatory liver biopsy or magnetic resonance imaging should be considered based upon the clinical conditions of the patient and the magnitude of ferritin increase. Subsequent monitoring is suggested for patients with continued RBC transfusions or hepatitis C infection. Transplant survivors with persistent IO must be considered for ferrochelation once engraftment is stable and immun-osuppressive treatment discontinued. Therapeutic phlebotomy (450-500 ml every 6 to 8 weeks) is advised as the treatment of choice in patients with more than 7 mg/kg dry weight liver iron; target iron status is serum ferritin within the normal laboratory range and transferrin saturation < 45%.

Therapeutic options for IO include phlebotomy and iron chelation therapy with chelator agents.

Phlebotomies

Phlebotomy may be considered as the treatment of choice for IO in patients with full recovery of erythropoiesis after HSCT. Based on the experience in patients with thalassemia, phlebotomy is a relatively inexpensive procedure that has been shown to effectively reduce iron burden alone or in combination with erythropoietin (EPO) support [73].

Indeed, data regarding the use of phlebotomies in HSCT recipients are scarce.

Busca *et al*. have evaluated the feasibility of phlebotomies in a small group of hematologic patients who received an allogeneic HSCT (35). Of 29 patients with evidence of IO based on LIC> 1000 mcg Fe/g wet weight, 19 (65%) were eligible for a phlebotomy program: 13 (68%) patients were able to complete the procedures reaching the target goal of ferritin level < 500 ng/mL. Recombinant EPO was used in 2 cases to facilitate planned phlebotomy. The median LIC was 1419 mg Fe/g ww (range, 1030-2384 mg Fe/g ww) before phlebotomy and 625 mg Fe/g ww (range, 250-1703 mg Fe/g ww) after completion of the program (P<0.001).

Venesections were performed in a group of 29 patients with post-HSCT IO, resulting in ferritin normalizetion in 24 out of 28 (86%) evaluable patients [39].

Kamble *et al*. described the outcome of 6 patients with IO exacerbating hepatic GVHD, who were treated with phlebotomy. EPO assisted phlebotomy led to normalization of liver function tests and of serum ferritin after a median of 11 months [75].

Majhail *et al*. reported the first study prospectively evaluating the management of IO in a small cohort of 16 allogeneic HSCT recipients with the use of phlebotomies or deferasirox (DFX): 8 patients underwent phlebotomies, 3 received DFX at the dose of 20 mg/Kg/day for 6 months and 5 patients chose observation only. Both treatments were reported to be well tolerated leading to decrease of serum ferritin level [76].

Iron-Chelating Therapy

Deferasirox (DFX)

A large retrospective study evaluated the efficacy of DFX in 80 patients with IO (defined as serum ferritin > 1000 ng/ml) before allogeneic HSCT [77]. Thirty-seven patients did not receive any treatment due to poor compliance, 43 patients received DFX treatment during the post-transplant period at the dose of 20-30 mg/Kg/day; the median day of treatment start was day+85 post-transplant and the median duration of DFX treatment was 122 days. The iron chelating treatment significantly reduced serum ferritin levels (2684 ng/ml, median value pre-treatment; 1097 ng/ml, median value post-treatment, $p<0.001$). More patients died in the non-treatment group (76%) as compared to patients in the treatment group (33%), leading to a significant better OS and DFS , although it should be underscored that more patients in the non-treatment group died of relapsed disease (13%) as compared to patients who were treated with DFX (5%). In this respect, we cannot exclude a selection bias of patients unable to receive DFX due to poor compliance that might simply reflect the high risk features of this group of patients.

Deferioxamine (DFO) and Deferiprone

Few studies have investigated the use of DFO for the treatment of IO in allogeneic HSCT recipients. Lee *et al*. reported the outcome of 43 patients who received iron-chelating agents before HSCT, and 37 of these were treated with DFO [78]. They found a correlation between the number of infusions and the reduction of ferritin levels [78]. The group of Pesaro described 15 thalassemic patients who received DFO before and after HSCT according to two schedules (from day -9 to day +60; from day -9 to day -2, and from day +28 to

day +60) at the dose of 40 mg/Kg/day as a 24-hour *i.v.* infusion [79]. The treatment did not influence the engraftment or the incidence of GVHD but led to a consistent reduction of ferritin levels [79].

The requirement of a subcutaneous or continuous intravenous administration of the drug may be considered a deterrent for a wider use of DFO in patients undergoing HSCT. A further obstacle to the use of DFO is the potential association between the occurrence of mucomycosis and the administration of DFO. In particular, mucorales have an absolute requirement for iron for their growth and survival, and DFO may function as a siderophore providing iron to the fungi [80].

To our knowledge there are no data regarding the use of deferiprone in allogeneic HSCT recipients; the occurrence of neutropenia potentially associated with the use of deferiprone represents a major hindrance to the administration of this agent in the allogeneic HSCT setting.

CONCLUSIONS

The great interest for IO in allogeneic HSCT recipients is witnessed by the large number of studies published during the most recent years. Nevertheless, many unresolved issues still remain.

The best tool to assess IO has not been identified: serum ferritin may be considered as the easiest way to estimate IO and the method most widely used worldwide. However, ferritin is a surrogate for IO and lacks of accuracy, while alternative methods, including SQUID and MRI, are expensive and, more importantly, are available in few Centers only. In addition, the best timing of assessment of IO has not defined precisely and usually relies on the policy in use at each transplant Center. Recent studies have questioned the association between IO and a poor patient outcome after HSCT. Since serum ferritin is an acute phase reactant, an elevation may simply mirror inflammatory conditions including an advanced disease phase that is well recognized to influence the outcome of hematologic patients receiving an allogeneic HSCT. On the other hand, several studies that quantified pre-HSCT IO with MRI-based hepatic iron concentration measurement did not show unequivocally an association between IO and overall survival or transplant-related mortality.

Phlebotomies may be considered as the standard of care for the treatment of IO, however only a minority of

HSCT recipients may benefit from this procedure due to the presence of concomitant anemia. Iron chelating agents, such as DFX, seem to have good safety profiles, however further investigations are required to confirm their efficacy in this setting.

Hopefully, future large prospective clinical trials will address these issues.

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