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Iron Overload in Patients Receiving Allogeneic Hematopoietic Stem Cell Transplantation: Quantification of Iron Burden by a Superconducting Quantum Interference Device (SQUID) and Therapeutic Effectiveness of Phlebotomy

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Iron overload (IO) is a known adverse prognostic factor in patients who undergo allogeneic hematopoietic stem cell transplantation (HSCT) for thalassemia and appears to play a similar role in patients with other hematologic disorders. The estimation of IO is based primarily on serum ferritin level; however, many confounding factors can result in ferritin overestimation, especially in HSCT recipients. The aim of the present study was to quantify IO after HSCT using a superconducting quantum interference device (SQUID), and to evaluate the impact of IO on hepatic function and infections. In addition, the feasibility of iron depletion was investigated. A total of 102 consecutive allogeneic HSCT recipients admitted to our outpatient department between December 2005, and December 2007, were analyzed. Primary diagnosis included acute leukemia/myelodysplastic syndrome in 61% of cases. Assessment of IO after HSCT included serum ferritin; in those with hyperferritinemia (ferritin $>$ 1000 ng/mL), liver iron concentration (LIC) was evaluated by SQUID magnetic susceptometry. Iron removal therapy was offered to patients with moderate IO (LIC 1000-2000 μg Fe/g wet weight [ww]) or severe IO (LIC $>$2000 μg Fe/g ww). Fifty-seven patients had a ferritin level $<$1000 ng/mL: the median time between HSCT and assessment of ferritin level was 1006 days (range, 93-5239 days), significantly different from the median time of 183 days (range, 78-2957 days) in the 45 patients with a ferritin level $>$1000 ng/mL. Out of 42 patients evaluated by SQUID, 29 had moderate to severe IO (median LIC value, 1493 μg Fe/g ww [range, 1030-3253]). In a multivariate analysis, a significant correlation was found between a ferritin level $>$1000 ng/mL and the presence of at least one abnormal liver function test (LFT) ORo = 6.8; 95% CI = 2.2-20.6). In addition, the rate of proven/probable invasive fungal disease was significantly higher in the patients with hyperferritinemia (13% vs 0%; $P = .006$). Nineteen of the 24 patients considered eligible for iron-depletion therapy underwent regular phlebotomy; 13 completed the program in a median of 287 days (range, 92-779 days), reaching the target of a ferritin level $<$ 500 ng/mL; LIC was significantly reduced (median, 1419 μg Fe/g ww to 625 μg Fe/g ww; $P < .001$) in 8 of the 9 patients who were revaluated by SQUID at the end of the iron-depletion program. In conclusion, the measurement of LIC obtained by SQUID documented the presence of moderate/severe IO in 69% of the patients with a high ferritin level. Our data showed that in HSCT recipients, high ferritin level is an independent risk factor for abnormal LFTs, and IO may be considered a potential risk factor for fungal infections. A phlebotomy program may be feasible in two-thirds of the patients who might benefit from iron depletion.

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INTRODUCTION

Iron overload (IO) is a well-established adverse prognostic factor in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) for thalassemia [1,2], and also appears to play a similar role in patients with other hematologic disorders [3-5]. Some studies indicate that IO may be considered a risk factor contributing to posttransplantation liver toxicity, veno-occlusive disease (VOD), increased susceptibility to infection, and graft-versus-host disease (GVHD) and may have a negative impact on survival as well [4,6,7].

Estimation of the iron burden is based primarily on ferritin as a surrogate marker for IO; however, many confounding factors can cause ferritin overestimation, particularly in HSCT recipients [8,9]. Thus, ferritin value alone is not an ideal measure of total body iron burden. A superconducting quantum interference device (SQUID), magnetic resonance imaging (MRI), and liver biopsy are more appropriate means of estimating liver iron content (LIC) [10-15]. On the other hand, whether iron depletion by either phlebotomy or chelation following allogeneic HSCT is feasible, and which patients might benefit from these procedures, remain unclear [13,16].

The aim of the present study was to quantify posttransplantation IO by means of serum ferritin, SQUID, and quantification of transfused iron in a cohort of 102 patients who underwent allogeneic HSCT. Further analyses included the impact of IO on hepatic function, infectious complications, and GVHD, as well as the feasibility and effect of an iron-depletion program.

PATIENTS AND METHODS

The study started in December 2005, and patient enrollment ended in December 2007. All patients undergoing allogeneic HSCT from January 1999 who were alive in continuous complete remission and had a minimum follow-up of 3 months were included in the trial and prospectively evaluated for posttransplantation IO. During this time frame, a total of 311 patients underwent allogeneic HSCT. Of these, 102 consecutive patients who fulfilled the inclusion criteria were included in the study; 136 patients who relapsed and 67 who died of transplantation-related complications, as well as 6 patients who were lost to follow-up, were not included in the analysis.

Underlying diseases included acute leukemia and myelodysplastic syndrome (MDS) in two-thirds of the patients. Table 1 summarizes demographic and clinical characteristics of the patients. The study was performed in accordance with the Helsinki Declaration and was approved by the Local Ethics Review committee. Written informed consent was obtained from all participating patients.

IO was initially assessed using different biomarkers, including serum iron, ferritin, and transferrin and transferrin saturation. For the purpose of this study, hyperferritinemia was defined as a serum ferritin level $>$ 1000 ng/mL [17-19]. Serial liver function tests (LFTs) were performed after transplantation at the time of iron status assessment, including aspartate aminotransferase (AST; reference range, 8-30 UI/L), alanine aminotransferase (ALT; reference range, 5-35 UI/L), gammaglutamyl transpeptidase (GGT; reference range, 8-35 UI/L), and alkaline phosphatase (ALP; reference range, 42-141 UI/L). Liver dysfunction was based on abnormally elevated LFT values on 2 or more occasions. All patients were evaluated for hepatitis B virus (HBV) and hepatitis C virus (HCV) status.

In patients with a serum ferritin level $>$1000 ng/mL in complete remission of their underlying disease, a quantitative measurement of LIC by SQUID was performed.

Data on the number of blood units transfused before SQUID evaluation were obtained from the blood bank of San Giovanni Battista Hospital. The iron content of the blood units collected in the blood bank between January 2005 and December 2008 (a total of 197,894 units) was calculated by multiplying the measured hemoglobin content by the volume of the unit by 3.4. The estimated mean iron content of our blood bags was 213 mg. The total mg of iron transfused per kg of recipient body weight was calculated, assuming that each unit of packed red blood cells (PRBCs) contained 213 mg of iron.

Medical charts were reviewed for GVHD status and the occurrence of bacteremias and invasive fungal infections between the day of transplantation and SQUID assessment.

A phlebotomy program was proposed to all patients with a ferritin value $>$ 1000 ng/mL and IO confirmed by SQUID (LIC $>$1000 μg Fe/g ww). With each phlebotomy, approximately 350-500 mL of whole blood was removed, depending on the patient's body weight. A complete blood count was analyzed before each phlebotomy, and the procedure was not performed in patients with a hemoglobin level $<$ 11 g/dL. Phlebotomies were repeated every 1-2 weeks until a serum ferritin level $<$ 500 ng/mL was measured. Iron removed in the single phlebotomy was calculated by multiplying the volume of the phlebotomy by hemoglobin concentration by 3.4. The total amount of iron removed was then calculated.

Statistical Analysis

The data were analyzed using SPSS version 16 for Windows (SPSS Inc, Chicago, IL). Normality was assessed by the Shapiro-Wilk test and exploratory data

Table 1. Patient and Transplantation Characteristics

Number of patients	102
Median age, years (range)	47 (21-67)
Underlying disease	
Acute myeloid leukemia	38 (37%)
Myelodysplastic syndrome	17 (17%)
Lymphomas	26 (25%)
Chronic myelogenous leukemia	6 (6%)
Myeloma	4 (4%)
Acute lymphoblastic leukemia	7 (7%)
Aplastic anemia	2 (2%)
Myelofibrosis	1 (1%)
Renal cell carcinoma	1 (1%)
Year of transplantation	
1999-2000	15
2001-2002	13
2003-2004	18
2005-2006	40
2007	16
Type of transplant	
Matched sibling donor	66 (65%
Matched unrelated donor	32 (31%)
Partially matched related donor	4 (4%)
Graft source	
Bone marrow	17 (17%)
Peripheral blood	84 (82%)
Cord blood	1 (1%)
Intensity of conditioning regimen	
Myeloablative	58 (57%)
Reduced-intensity	44 (43%)
Conditioning regimen	
TBI 1200 cGy + Cy	23 (22%)
TBI 200 cGy + fludarabine	24 (23%)
TBI 200 cGy	2 (2%)
Thiotepa-Cy	38 (37%)
Busulfan-containing regimen*	6 (6%)
Other	9 (10%)

TBI indicates total body irradiation; Cy, cyclophosphamide.
*Four patients received busulfan 16 mg/kg by oral administration; 2 patients received busulfan 12.8 mg/kg i.v.

analysis. Data are expressed as median and range for continuous variables and frequencies (%) for categorical variables. Differences between groups were assessed using the Mann-Whitney test, whereas associations between categorical variables were evaluated using Fisher's exact test. Univariate and multivariate logistic regression models were used to analyze the effects of some relevant variables (ie, hepatitis, chronic GVHD [cGVHD], disease status, timing of ferritin assessment, and hyperferritinemia) on liver dysfunction. Time of ferritin assessment was included in the models as a categorical variable using tertiles. The correlation between ferritin and LIC with the amount of iron transfused was assessed by Spearman's correlation coefficient. All statistical tests were 2-sided and significant for $P < .05$.

RESULTS

Iron Overload

The median serum ferritin level was 837 ng/mL (range, 18-11,110 ng/mL). Twenty-eight patients had a ferritin value within the normal range (reference range, 25-340 ng/mL), and 74 patients (72%) had an above-normal ferritin value. Overall, 45 of the 102 patients (44%) had a serum ferritin level > 1000 ng/mL. IO assessment was performed at a median time of 578 days after HSCT (range, 78-5293 days). In the cohort of patients with hyperferritinemia, the median time from HSCT to ferritin assessment was 183 days (range, 78-2957 days), significantly shorter than the median time of 1006 days (range, 93-5239 days) in the 57 patients with a ferritin level below the threshold of 1000 ng/mL ($P < .0001$) (Table 2). Hyperferritinemia was detected in 59% (32/54) of the patients with acute leukemia or MDS, compared with 27% (13/48) of those with a different diagnosis ($P = .002$).

A pre-HSCT serum ferritin value was available for 63 patients. Thirty-three of these patients had a ferritin level below the threshold of 1000 ng/mL (median, 529 ng/mL; range, 15-991 ng/mL); 10 of these patients exhibited hyperferritinemia post-HSCT. Thirty of the patients had a ferritin level ≥ 1000 ng/mL (median, 1963 ng/mL, range, 1083-18,290 ng/mL); 25 of these maintained a high ferritin level post-HSCT, while 5 exhibited a spontaneous decrease in ferritin level.

LIC (μg Fe/g ww) was evaluated by SQUID in 42 of the 45 patients with a ferritin level > 1000 ng/mL. IO was not assessed by SQUID in 2 patients who developed disease recurrence and in 1 patient who died shortly after ferritin assessment. Quantification of LIC by SQUID was obtained at a median of 49 days (range, 3-338 days) after the assessment of serum ferritin. Overall, the median LIC value was 1298 μg Fe/g ww (range, 250-3253 μg Fe/g ww), corresponding to 7.6 mg Fe/g dry weight (range, 1.5-19 mg Fe/g dw). Twenty patients (48%) had moderate IO (LIC 1000-2000 μg Fe/g ww), and 9 (21%) had severe IO (LIC > 2000 μg Fe/g ww). Five patients (12%) had normal a LIC value (< 400 μg Fe/g ww) despite a high serum ferritin level; 4 of these patients had GVHD involving the liver (n = 2), skin (n = 1), or oral mucosa and eye (n = 1), and 1 patient had severe CNS infection. The patients with an LIC >1000 μg/g ww received a median of 32 PRBCs (range, 7-101) and a median of 116 mg/kg of iron (range, 12-316) before SQUID evaluation. The biochemical parameters and transfusion data are summarized in Table 2.

We found a statistically significant correlation between serum ferritin level and the amount of iron transfused, defined as mg of iron transfused per kg of recipient body weight ($\rho = 0.709$; $P < .001$). The correlation between amount of iron transfused and LIC as measured by SQUID was statistically significant only in male recipients ($\rho = 0.510$; $P = .018$).

Effect of Iron Overload on HSCT Outcome

At the time of IO assessment, LFT values were above the upper limit of normal in 69 of the 102 patients. Thirty-one of the 57 patients (54%) with

Table 2. Iron Burden Measurements and Liver Function Tests after Transplantation

	Patients with Hyperferritinemia (Ferritin ≥ 1000 ng/mL)	Patients with Ferritin < 1000 ng/mL	*P*
Number of patients	45	57	
Serum iron, μg/dL	136 (33-378)	82 (12-348	<.001*
Transferrin saturation, %	56 (13-110)	31 (5-101)	<.001*
Ferritin, ng/mL	1609 (1027-11110)	346 (6-974)	<.001*
Time to ferritin assessment, days	183 (78-2957)	1006 (93-5239)	<.001*
LIC, ngFe/g ww)	1311 (298-2476)	-	
LIC, ng/g dw)	7.6 (1.7-16.4)	-	
Total iron transfused, mg/kg	107.9 (12.1-316.3)	32.2 (4.7-209.8)	<.001*
Patients with LFT above UNL, n (%)			
AST	25 (55%)	19 (33%)	.028†
ALT	32 (71%)	19 (33%)	<.001†°
GGT	31 (69%)	21 (37%)	.001†°
ALP	7 (15%)	7 (12%)	.773†°
AST, U/L	35 (14-291)	26 (15-288)	.091*
ALT, U/L	50 (9-904)	28 (6-594)	.001*
GGT, U/L	72 (8-1262)	28 (8-302)	<.001*
ALP, U/L	92 (34-546)	83 (44-324)	.345*

AST indicates aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gammaglutamyl transpeptidase; ALP, alkaline phosphatase.
Data are reported as median (range) unless specified otherwise.
*Mann-Whitney test.
†Fisher's exact test.

a post-HSCT ferritin level <1000 ng/mL had at least 1 abnormal LFT value, compared with 38 of the 45 patients (84%) with post-HSCT hyperferritinemia ($P = .001$). Thirty-five percent of the patients with a serum ferritin level <1000 ng/mL (20/57) and 71% of those with hyperferritinemia (32/45) had 2 or more abnormal LFT values ($P < .001$). Median GGT and ALT values were significantly higher in the patients with hyperferritinemia, whereas there was only a trend toward higher AST values in the patients with hyperferritinemia (Table 2).

In both univariate and multivariate analyses, a significant association was found between ferritin level > 1000 ng/mL and liver dysfunction, defined by the presence of at least 1 abnormal LFT value on 2 or more occasions. This association is statistically significant even when the varying times of ferritin assessment are included in the multivariate model (Table 3).

Three patients with a ferritin level < 1000 ng/mL and 4 patients with hyperferritinemia were HBV-positive. Two patients with a ferritin level < 1000 ng/mL and 1 patient with hyperferritinemia were HCV-positive.

Severe acute GVHD (aGVHD) grade II-IV occurred in 24 patients. Eight of these patients (33%) had a ferritin level > 1000 ng/mL, compared with 37 of 78 patients (47%) with aGVHD grade 0-I ($P = .326$). No significant correlation was found between hyperferritinemia and the presence of cGVHD ($P = .685$).

No patient included in the study presented with clinical signs of VOD. Nine 9 patients who died of a transplantation-related complication (n = 7) or disease recurrence (n = 2) during the first 3 months post-HSCT had evidence of VOD. These patients were not included in the analysis.

Information on the occurrence of bacteremia was available for 94 patients. Overall, 12 patients had a positive blood culture, including 7 patients (16%) with hyperferritinemia and 5 patients (10%) with a serum ferritin level < 1000 ng/mL ($P = .455$). Isolates were *Staphilococcus coagulase negative* (n = 5), *Staphilococcus aureus* (n = 2), *Corynebacterium jeikeium* (n = 1), *Escherichia coli* (n = 2), *Pseudomonas aeruginosa* (n = 1), and *Stenotropomonas maltophilia* (n = 1). One patient with hyperferritinemia, but a normal LIC developed a bloodstream infection.

Invasive fungal disease (IFD) developed in 6 patients (13%) with hyperferritinemia but in none of the patients with a serum ferritin level < 1000 ng/mL ($P = .006$). Five of these cases were defined as probable pulmonary aspergillosis according to established criteria [20]; 1 patient had invasive candidiasis of the esophagus. The median time between HSCT and the diagnosis of IFD was 12 days (range, 0-110 days), and the median time from diagnosis of IFD to the assessment of serologic ferritin level was 14 months (range, 2-30 months). One patient with hyperferritinemia but a normal LIC developed IFD. In 5 of the 6 patients with IFD, a serum ferritin measurement was available before the diagnosis of IFD was made; in 4 of these cases, the ferritin level was > 1000 ng/mL and in the remaining case the level was 991 ng/mL.

Overall, 16 patients died. Of the 9 patients who died of transplantation-related complications, 6 had hyperferritinemia; of the 7 who died of recurrence of the underlying disease, 6 had hyperferritinemia.

Effect of Phlebotomy

Of the 29 patients with LIC > 1000 μg Fe/g ww, 3 were considered ineligible for an iron-depletion

Table 3. Impact of Hyperferritinemia on Liver Function

Variable	Patients with liver dysfunction*	Univariate OR (95% CI)	Multivariate OR (95% CI)
Hepatitis			
No (n = 92)	49 (53%)	1	-
Yes (n = 10)	8 (80%)	3.5 (0.7-17.4)	-
Chronic GVHD			
Absent (n = 60)	30 (50%)	1	-
Limited (n = 28)	17 (61%)	1.5 (0.6-3.8)	-
Extensive (n = 14)	10 (71%)	2.5 (0.7-8.9)	-
Disease status			
Early (n = 56)	34 (61%)	1	-
Advanced (n = 46)	23 (50%)	0.6 (0.3-1.4)	-
Timing of ferritin assessment (days after HSCT)			
< 192 (n = 34)	17 (50%)	1	1
193-1006 (n = 34)	19 (56%)	1.3 (0.5-3.3)	2.7 (0.8-8.7)
> 1007 (n = 34)	21 (62%)	1.6 (0.6-4.2)	5.4 (1.5-20.1)
Hyperferritinemia			
< 1000 ng/mL (n = 57)	31 (54%)	1	1
> 1000 ng/mL (n = 45)	38 (84%)	3.1 (1.3-7.0)	6.8 (2.2-20.6)

GVHD indicates graft-versus-host-disease; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gammaglutamyl transpeptidase; ALP, alkaline phosphatase.
*Patients with at least one abnormal liver function test (AST, ALT, GGT, or ALP).

program because of either recurrence of the underlying malignancy (n = 2) or poor general condition (n = 1). Two patients who exhibited a spontaneous normalization of ferritin level were not considered for iron depletion; at the time of ferritin assessment, 1 of these patients had mildly elevated LFT values consistent with hepatic GVHD, and the other had concomitant cytomegalovirus (CMV) and urinary tract infections (UTIs).

Five patients (17%) were not eligible for a phlebotomy program. Three of these patients were treated with deferasirox, 1 patient was treated with desferrioxamine because of a low hemoglobin level, and 1 patient received no treatment because of his poor general condition. Deferasirox was admistered at a dose of 10 mg/kg/day in all patients but 1, who received 20 mg/kg.

Nineteen patients (65%) with LIC > 1000 μmg Fe/g ww were treated by regular phlebotomy. The venesections were initiated between day +210 and day +3488 post-HSCT (median, day +757). Recombinant erythropoietin was used in 2 cases to facilitate planned phlebotomy. For 1 patient, the program is still ongoing, whereas 13 patients completed the program, reaching the target goal of ferritin level < 500 ng/mL after a median of 16.5 phlebotomies (range, 8-38) and 2392 mg of iron removed (range, 1160-5510 mg). The median duration of the phlebotomy program was 287 days (range, 92-779 days).

The effect of phlebotomy on IO is illustrated in Figure 1. One of the 9 patients evaluated by SQUID showed no remarkable reduction in LIC despite serum ferritin normalization. Interestingly, patients who completed the phlebotomy program exhibited significantly improved LFT values. Eight of the 12 patients with abnormal LFT values before the initiation of phlebotomy had normalization of at least 1 test at the end of the program. The median AST value was 57 U/L (range, 46-86 U/L) before phlebotomy and 27 U/L (range, 21-39 U/L) at the end of treatment ($P = .027$). The median ALT value was 98 U/L (range, 50-140 U/L) before phlebotomy and 28 U/L (range, 20-51 U/L) at the end of treatment ($P = .007$). The median GGT value was 212 U/L (range, 58-1262 U/L) before phlebotomy and 51 U/L (range, 23-237 U/L) at the end of treatment ($P = .017$). Five patients discontinued the phlebotomy program. Two of these patients were switched to deferasirox because of the presence of hypotension (1 case) and progressive anemia (1 case); 2 patients who relapsed and 1 patient who died of GVHD received no additional iron chelation.

Overall, 5 patients received deferasirox for a median duration of 270 days (range, 37-425 days). One patient discontinued deferasirox because of the occurrence of transplantation-related complications

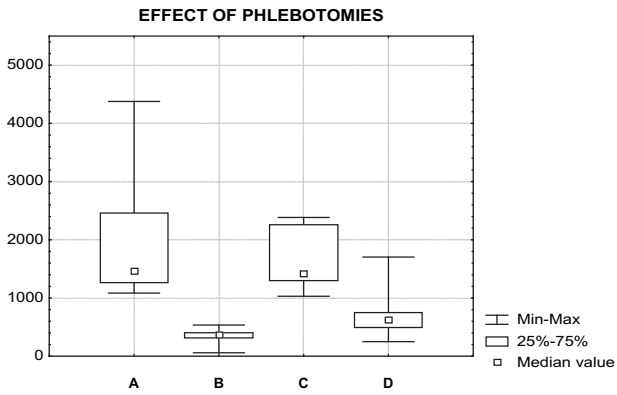

Figure 1. The impact of phlebotomy on serum ferritin level and LIC. The median ferritin level was 1459 ng/mL (range, 1084-4376 ng/mL) before phlebotomy (column A), compared to 373 ng/mL (range, 59-535 ng/mL) after completion of the program (column B) ($P < .001$). The median LIC was 1419 mg Fe/g ww (range, 1030-2384 mg Fe/g ww) before phlebotomy (column C) and 625 mg Fe/g ww (range, 250-1703 mg Fe/g ww) after completion of the program (column D) ($P < .001$).

(severe hemolytic anemia in one case) and another patient did so because of an increase in serum creatinine level concomitant with the higher dose of deferasirox (20 mg/kg). Two patients completed the treatment, reaching ferritin levels of 356 ng/mL and 885 ng/mL, respectively. One patient is currently undergoing the treatment. Overall, deferasirox was well tolerated, with transient mild gastrointestinal disturbances reported in 2 cases and cutaneous rash reorted in 1 case. No cases of drug-related cytopenia were noted.

DISCUSSION

IO is a relevant but often underestimated complication in HSCT recipients, primarily resulting from the paucity of tools available for a reliable diagnosis of iron burden. Given these considerations, 2 major factors are of particular clinical interest: the frequent misleading diagnoses, including GVHD and drug-related toxicity, and the need to extensively evaluate and validate all therapeutic approaches [10,16].

In the present study, we assessed the prevalence of IO in 102 HSCT recipients at varying time intervals from transplantation, using serum ferritin level to indirectly estimate IO and SQUID to directly measure LIC. Overall, 44% of our patients exhibited evidence of IO based on serum ferritin level; this relatively high prevalence of IO is in agreement with the 58% reported by Rose et al [11] and the 32% reported by Majhail et al [13]. The quantification of LIC by SQUID confirmed the presence of moderate to severe iron burden in two-thirds of the patients with hyperferritinemia. To the best of our knowledge, the assessment of iron burden by SQUID in HSCT recipients has been reported in only 1 previous study [14]. In the present study, 5 patients had normal LIC despite a high ferritin level, and 2 patients experienced spontaneous normalization of ferritin level. We can hypothesize that the significant inflammatory status associated with the comorbidities present in these patients at the time of ferritin assessment (ie, GVHD in 5 cases and severe infection in 2 cases) might be responsible for the misleading serum ferritin values.

A significant correlation between LIC and the amount of iron transfused was seen only in male recipients, possibly because of the variable physiological blood loss in females. Alternatively, the study's small sample size may account for this finding. In addition, it is well known that transfusions are not the only cause of IO in patients with hematologic malignancies and those undergoing HSCT. Other possible causes of IO include ineffective erythropoiesis leading to decreased iron utilization, increased intestinal absorption of iron due to mucositis and GVHD, and abnormal hepcidin regulation [10,16,21]. Recently, it also has been hypothesized that alloreactive T cell-dependent signals may induce dysregulation of intestinal iron absorption [22]. Considering that liver biopsy with estimation of LIC is the reference method for measuring hepatic iron stores [23], but is an invasive procedure and may not be feasible in thrombocytopenic patients, we can hypothesize that body iron stores in patients after HSCT are optimally assessed through a comprehensive study of iron burden, including the evaluation of serum ferritin level, amount of iron transfused, and LIC by SQUID or MRI.

The median time interval from HSCT to ferritin measurement was significantly shorter in the patients with hyperferritinemia compared with those with a ferritin level < 1000 ng/mL (183 days vs 1006 days), suggesting that a physiological decrease in ferritin level may occur post-HSCT, as has been reported in previous studies [24,25]. Alternatively, the high ferritin level might reflect the proinflammatory condition usually observed during the first 6 months post-HSCT, in which GVHD and infectious complications play a crucial role. In this respect, it is noteworthy that 9 of 13 patients with hyperferritinemia, but LIC < 1000 μg Fe/g ww underwent the biochemical assessment within 6 months after HSCT, suggesting that, at least in these patients, the ferritin value might not represent a true condition of IO.

Liver dysfunction is a very common complication of allogeneic HSCT, occurring in 57%-80% of patients [8,9,15,26]. GVHD, drug toxicity, VOD, and infections are the most common factors involved in the etiology of liver dysfunction in allogeneic HSCT recipients, although more recently IO has emerged as an important cause of abnormal LFT values [9,15,16,26], particularly in the intermediate/late post-transplantation period (> 30 days post-HSCT). Our results support the latter observation; 38 of 69 patients with abnormal LFT values had a high serum ferritin level, and multivariate analysis confirmed a significant correlation between hyperferritinemia and liver dysfunction. It is noteworthy that this correlation reached statistical significance, even when the varying time to ferritin estimation was included in the statistical model, thereby minimizing the potential effects of other confounding factors, such as regimen-related toxicity, GVHD, and infections, which usually are more relevant during the first 6 months post-HSCT.

ALT and GGT were the LFT values that most frequently exceeded the upper limit of normal in the patients with hyperferritinemia, and they reached a significantly higher median peak level in the patients with high ferritin levels. To the best of our knowledge, these findings have not previously reported in the literature. Given our findings, transplantation clinicians should be aware that elevated ALT and particularly GGT might reflect the damage of iron burden, although unfortunately these parameters are not specific for, but rather are merely suggestive, of hepatic IO.

Our analysis has confirmed that IO is a potential risk factor for IFD, as has been reported previously [27,28], although this finding should be confirmed in larger prospective clinical trials. In this respect, it would be of interest to evaluate whether iron depletion is able to restore the capacity of the host to combat infection. Alternatively, the possibility that iron starvation might slow the growth of fungal hyphae is intriguing [29,30].

Phlebotomy is the treatment of choice for patients who develop IO after HSCT. Based on European Group for Blood and Marrow Transplantation recommendations, long-term HSCT survivors with IO documented by LIC > 7 mg Fe/g dw should be treated with phlebotomy and chelation therapy [31]. Several studies have documented the feasibility and efficacy of a phlebotomy program in HSCT recipients. Butt et al [25] reported that ferritin levels fell more rapidly in autograft recipients who underwent venesection than in those who did not undergo venesection. Rose et al [11] reported the results of phlebotomy in 29 patients with abnormal LIC estimated by MRI after allogeneic HSCT. Ferritin level normalized in 86% of the evaluable patients, and 62% of the patients had normalized hepatic biology after phlebotomy. Tomas et al [9] described 26 patients with chronic liver dysfunction after allogeneic HSCT who were included in a iron-depletion program. Of these patients, 23 underwent venesection at a median of 539 days after HSCT, and improved LFT values were documented in 21 of them. Our findings confirm that iron depletion by phlebotomy significantly reduces both ferritin level and LIC as assessed by SQUID. In addition, phlebotomy helped ameliorate abnormal LFT values, as demonstrated by the fact that 67% of the patients exhibited normalized LFT values after completing the program. Nevertheless, a consistent proportion of HSCT recipients may not be eligible for phlebotomy because of coexisting anemia. These patients may benefit from treatment with an iron-chelating agent; however, few studies have analyzed the feasibility and the effectiveness of these agents following HSCT [30,32,33]. In our study, 5 patients were treated with the oral chelator deferasirox, and 2 of these patients discontinued therapy because of drug-related side effects, including gastrointestinal disturbance and renal insufficiency. Unfortunately, given the small number of cases included in the present analysis, a meaningful conclusion regarding the efficacy of deferasirox in the setting of post-HSCT IO cannot be drawn.

In conclusion, the measurement of LIC by SQUID documented the presence of moderate to severe IO in 69% of our HSCT recipients with high ferritin levels. Estimation of iron status by measuring serum ferritin level seems to be more precise at least 6 months after HSCT, avoiding the possible interference of inflammatory factors on ferritin measurement. Our data show that high ferritin level is an independent risk factor for the occurrence of abnormal LFT values, and that IO may be considered a potential risk factor for fungal infection. A phlebotomy program was feasible in two-thirds of the patients who might benefit from iron depletion and effectively normalized LFT values in 67% of the patients. Whether alternative strategies, including oral iron-chelating agents, may be useful in this setting merits further investigation. In addition, larger prospective clinical trials are needed to verify the applicability of phlebotomy even in early stages post-HSCT.

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