4. Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med*. 1995; 333:1540-1545.
5. William BM, Loberiza FR Jr, Whalen V, et al. Impact of conditioning regimen on outcome of 2-year disease-free survivors of autologous stem cell transplantation for Hodgkin lymphoma. *Clin Lymph Myel Leuk*. 2013;13:417-423.
6. Xie H, Griskevicius L, Stahle L, et al. Pharmacogenetics of cyclophosphamide in patients with hematological malignancies. *Eur J Pharm Sci*. 2006;27:54-61.
7. Takada K, Arefayene M, Desta Z, et al. Cytochrome P450 pharmacogenetics as a predictor of toxicity and clinical response to pulse cyclophosphamide in lupus nephritis. *Arthritis Rheum*. 2004;50:2202-2210.
8. Raccor BS, Claessens AJ, Dinh JC, et al. Potential contribution of cytochrome P450 2B6 to hepatic 4-hydroxycyclophosphamide formation in vitro and in vivo. *Drug Metab Dispos*. 2012;40:54-63.
9. Thorn CF, Lamba JK, Lamba V, et al. PharmGKB summary: very important pharmacogene information for CYP2B6. *Pharmacogenet Genom*. 2010;20:520-523.
10. Cheson BD. New staging and response criteria for non-Hodgkin lymphoma and Hodgkin lymphoma. *Radiol Clin North Am*. 2008;46: 213-223. vii.
11. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
12. Lin DY. Non-parametric inference for cumulative incidence functions in competing risks studies. *Stat Med*. 1997;16:901-910.
13. Cox DR. Regression models and life tables. *J R Stat Soc B*. 1972;34: 187-220.
14. Holm S. A simple sequentially rejective multiple test procedure. *Scand J Stat*. 1979;6:65-70.
15. Helsby NA, Hui CY, Goldthorpe MA, et al. The combined impact of CYP2C19 and CYP2B6 pharmacogenetics on cyclophosphamide bioactivation. *Br J Clin Pharmacol*. 2010;70:844-853.
16. Rocha V, Porcher R, Fernandes JF, et al. Association of drug metabolism gene polymorphisms with toxicities, graft-versus-host disease and survival after HLA-identical sibling hematopoietic stem cell transplantation for patients with leukemia. *Leukemia*. 2009;23:545-556.
17. Black JL, Litzow MR, Hogan WJ, et al. Correlation of CYP2B6, CYP2C19, ABCC4 and SOD2 genotype with outcomes in allogeneic blood and marrow transplant patients. *Leuk Res*. 2012;36:59-66.
18. Melanson SE, Stevenson K, Kim H, et al. Allelic variations in CYP2B6 and CYP2C19 and survival of patients receiving cyclophosphamide prior to myeloablative hematopoietic stem cell transplantation. *Am J Hematol*. 2010;85:967-971.
19. Zanger UM, Klein K. Pharmacogenetics of cytochrome P450 2B6 (CYP2B6): advances on polymorphisms, mechanisms, and clinical relevance. *Front Genet*. 2013;4:24.
20. Ekhart C, Doodeman VD, Rodenhuis S, et al. Influence of polymorphisms of drug metabolizing enzymes (CYP2B6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, GSTA1, GSTP1, ALDH1A1 and ALDH3A1) on the pharmacokinetics of cyclophosphamide and 4-hydroxycyclophosphamide. *Pharmacogenet Genom*. 2008;18:515-523.
21. Helsby NA, Tingle MD. Which CYP2B6 variants have functional consequences for cyclophosphamide bioactivation? *Drug Metab Dispos*. 2012;40:635-637.
22. Salar A, Sierra J, Gandarillas M, et al. Autologous stem cell transplantation for clinically aggressive non-Hodgkin's lymphoma: the role of preparative regimens. *Bone Marrow Transplant*. 2001;27:405-412.
23. Johnson GG, Lin K, Cox TF, et al. CYP2B6*6 is an independent determinant of inferior response to fludarabine plus cyclophosphamide in chronic lymphocytic leukemia. *Blood*. 2013;122:4253-4258.

Association between Plasma Endothelin-1, Transforming Growth Factor-β, Fibroblast Growth Factor, and Nitric Oxide Levels and Liver Injury in Hematopoietic Stem Cell Transplantation Recipients with Persistent Iron Overload after Transplantation

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ABSTRACT

Graft-versus-host disease, iron overload, and infections are the major causes of liver dysfunction in allogeneic hematopoietic stem cell transplantation (AHSCT) recipients. We investigated the relationship between serum iron parameters and the levels of transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), endothelin-1 (ET-1), and nitric oxide (NO) as predictors of chronic liver injury in 54 AHSCT recipients who survived at least a year after transplantation. Serum samples from patients were obtained for the evaluation of ET-1, TGF-β, FGF, NO, and nontransferrin bound iron at the first year follow-up visit using commercially available ELISA kits. Patients were categorized depending on serum ferritin and transferrin saturation levels. The parameters were compared between the groups, and survival analysis was also performed. Most of the AHSCT recipients (81.5%) were in complete remission during the study. After a median follow-up time of 73 months (range, 13 to 109 months), 72.2% of the patients were alive. Mean serum levels of ET-1, NO, TGF-β, and FGF were 81.54 ± 21.62 μmol/mL, 31.82 ± 26.42 μmol/mL, 2.56 ± 0.77 ng/mL, and 50.31 ± 32.69 pg/mL, respectively. Nineteen patients (35.2% of the cohort) had serum ferritin levels higher than 1000 ng/mL. Mean

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serum levels of ET-1, NO, TGF-β, and FGF were similar in patients with serum ferritin levels below or above 1000 ng/mL ($P > .05$). Serum ferritin levels were positively correlated with serum alanine aminotransferase ($r = .284$, $P = .042$) and γ-glutamyl transferase ($r = .271$, $P = .05$) levels and were negatively correlated with serum albumin levels ($r = .295$, $P = .034$). There was a significant positive correlation between serum transferrin saturation and alanine aminotransferase levels ($r = .305$, $P = .03$). Serum ET-1 level was positively correlated with alkaline phosphatase levels ($r = .304$, $P = .026$). In univariate Cox regression analysis serum levels of iron parameters, ET-1, NO, TGF-β, and FGF did not have an impact on overall survival ($P > .05$). The probability of progression-free survival was also similar in patients with ferritin levels above or below 1000 ng/mL ($P = .275$). The probability of survival was similar in patients with transferrin saturation ≥70% and <70% ($P > .05$). Serum iron parameters showed a positive correlation with liver injury. However, there was no correlation between fibrogenic cytokines and liver transaminases. Our results suggest that iron overload at least with the current levels of ferritin might have a relatively benign course. Prospective randomized trials will guide the actual role of iron chelation in the post-transplantation setting.

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INTRODUCTION

Liver dysfunction is frequently seen in allogeneic hematopoietic stem cell transplantation (AHSCT), and chronic graft-versus-host disease (GVHD), iron overload (IO), and infections per se or in combination are responsible for most cases [1]. These factors, directly or indirectly through the induction of cytokines, might lead to chronic liver injury [2]. Repeated red cell transfusions, increased gastrointestinal iron absorption due to inflammatory peritransplant conditions caused by chemotherapy and GVHD, and ineffective erythropoiesis are the major causes of IO in HSCT recipients [3,4].

Excess iron results in the appearance of nontransferrin-bound iron (NTBI), which mediates direct tissue injury and organ toxicity. NTBI corresponds to iron unbound to transferrin, heme, or ferritin iron. The main form of NTBI as iron (III) citrate is efficiently taken up by the liver [4,5]. Excess iron can damage tissues by catalyzing the conversion of superoxide and hydrogen peroxide to free radical species by attacking cellular membranes, proteins, and DNA [6].

The impact of pretransplantation IO on survival and treatment-related complications has been reviewed extensively [4,7-12]. Although a previous report claims that IO is persistent up to 2 years and preserves its adverse prognostic significance [13], the clinical and prognostic significance of persistent IO post-transplantation has not yet been fully clarified. Hepatic IO was previously found as a major cause of hepatic injury post-AHSCT, and serum ferritin was found to be correlated to the histological grade of iron in the hepatocytes [14]. A similar prospective observational study indicates a strong correlation between liver iron content, serum ferritin level, and the number of transfusions a year after transplantation [15].

Despite the increased frequency and the well-established early effects, the impact of IO on the long-term morbidity and/or mortality of HSCT has not yet been established. The aim of our study was to analyze the incidence and extent of IO after HSCT. We also investigated the interaction between the serum iron parameters and serum levels of transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), endothelin-1 (ET-1), and nitric oxide (NO) as predictors of liver injury in AHSCT recipients with persistent IO.

METHODS

Fifty-four consecutive adult AHSCT recipients who lived at least 1 year after HSCT were included in this study. Serum samples are routinely stored at the 1-year follow-up visit per institutional practice. Written informed consent was obtained from all patients before transplantation for a fraction of their blood samples obtained for routine tests at follow-up visits to be stored for any further potential study per institutional practice. Forty-one patients had their samples already stored at the 1-year follow-up, whereas 13 patients had their serum samples collected prospectively after approval of the Institutional Review Board and obtaining informed consents from the patients. AHSCT recipients who were conditioned with a myeloablative regimen received cyclosporine A from day −1 and methotrexate on days +1, +3, +6, and +11 for the prevention of GVHD. Patients conditioned with a nonmyeloablative regimen received cyclosporine A from day −1 and mycophenolate mofetil from day +1 as GVHD prophylaxis.

Serum samples for the analysis of ET-1, TGF-β, FGF, NO, and NTBI were obtained at the first year post-transplant visit and stored at −80°C until analysis. Serum ferritin and transferrin saturation (TS) levels, acute phase reactant levels, serum albumin, and alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transferase, and alkaline phosphatase were studied using a modular autoanalyzer (Cobas Integra 800; Roche, Indianapolis, IN). Serum NTBI levels were measured using the method described previously by Gosriwatana et al. [16]. ET-1, NO (Cayman Company, Ann Arbor, MI), TGF-β, and FGF (Invitrogen, Carlsbad, CA) levels were measured using commercially available ELISA kits. Patients were grouped according to the serum ferritin levels as <1000 ng/mL versus ≥1000 ng/mL and for TS as <70% versus ≥70%. National Institutes of Health criteria were used to categorize acute and chronic GVHD [17]. Disease stage was categorized as early, intermediate, and late according to the European Group for Blood and Marrow Transplantation risk score [18].

Statistical Analysis

The statistical evaluation of the data was performed using SPSS software, version 21 (SPSS Inc, Chicago, IL). Differences between groups were analyzed by the Mann-Whitney U test. Correlation between variables were evaluated using Pearson or Spearman's correlation tests where appropriate. A value of $P < .05$ was considered to represent a statistically significant difference. Survival analysis was carried out by using Kaplan-Meier and log-rank tests.

RESULTS

Fifty-four consecutive AHSCT recipients (17 female, 37 male) with a median age of 25 years (range, 16 to 53) who lived at least 1 year after HSCT were included in the study. Conditioning regimens were myeloablative in 35 patients (64.8%) and nonmyeloablative in 19 patients (35.2%). Demographic, clinical, and transplantation-related characteristics of patients are summarized in Table 1.

Eight patients (14.8%) had a history of sinusoidal obstruction syndrome early after transplantation. Thirteen patients (24.1%) were still on immunosuppressive treatment during analysis.

European Group for Blood and Marrow Transplantation risk score was <3 in 35 of 54 patients (64.8%) and ≥3 in 19 of 54 patients (35.2%). Forty-four patients (81.5%) were in complete remission at the time of analysis. All patients were transfusion independent, except 4 patients with relapsed disease. The incidence of acute GVHD, including both classic acute and late-onset acute, was 27.8% and chronic GVHD, including both classic and overlap syndrome, was 22.2%.

Thirteen of 54 patients (24.1%) had persistent GVHD at the time of analysis. Liver involvement as a part of acute and chronic GVHD was detected in 21 of 54 patients (38.9%). Severity of chronic GVHD in 12 patients was mild in 1 (9.0%), moderate in 6 (50.0%), and severe in 5 patients (41%). Thirteen of 54 patients (24.1%) were on immunosuppressive treatment with cyclosporine A at the time of analysis. After a median follow-up of 73 months (range, 13 to 109), 39 of 54 patients (72.2%) were alive. The cause of death was primary disease in 12 of 54 patients (22.2%) and transplant-related mortality in 3 of 54 patients (5.6%).

Mean serum ferritin, NTBI levels, and TS in all patients were 1036.21 ± 1572.12 ng/mL, 6.37 ± 5.71 μg/dL, and 41.77% ± 24.04%, respectively. Mean serum levels of ET-1, NO, TGF-β, and FGF were 81.54 ± 21.62 μmol/mL, 31.82 ± 26.42 μmol/mL, 2.56 ± .77 ng/mL, and 50.31 ± 32.69 pg/mL, respectively. In 19 of 54 patients (35.2%), which constitutes the high ferritin group, serum ferritin levels were ≥1000 ng/mL 1 year after HSCT.

Correlation Analysis of Serum Iron Parameters and ET-1, NO, TGF-β, and FGF Levels

Serum ferritin levels showed significant positive correlation with serum NTBI levels ($r = .485$, $P < .001$) and TS ($r = .597$, $P < .001$). There was a significant negative correlation between serum ferritin and ET-1 levels ($r = -.271$, $P = .05$). Serum iron parameters, including ferritin, TS, and NTBI, did not show a correlation with serum NO ($r = .197$, $P = .161$; $r = .174$, $P = .213$; and $r = .032$, $P = .819$, respectively), TGF-β ($r = .050$, $P = .724$; $r = .053$, $P = .709$; and $r = .082$, $P = .556$, respectively), and FGF ($r = .110$, $P = .436$; $r = .047$, $P = .739$; and $r = .045$, $P = .744$, respectively). There was a significant negative correlation between serum ET-1 and NO levels ($r = -.366$, $P = .006$).

Correlation Analysis with Liver Function Test

Serum ferritin levels were positively correlated with serum alanine aminotransferase ($r = .284$, $P = .04$) and γ-glutamyl transferase ($r = .271$, $P = .05$) levels and were negatively correlated with serum albumin levels ($r = .295$, $P = .03$). Serum ferritin and NTBI levels were positively correlated with prothrombin time ($r = .662$, $P = .003$ and $r = .567$, $P = .01$, respectively). There was a significant positive correlation between serum TS and alanine aminotransferase levels ($r = .305$, $P = .03$). Serum ET-1 level was positively correlated with alkaline phosphatase levels ($r = .304$, $P = .03$).

Comparison of ET-1, NO, TGF-β, and FGF Levels between High and Low Ferritin and TS Groups

Mean serum levels of ET-1, NO, TGF-β, and FGF were similar in the high and low ferritin groups ($P > .05$). Mean levels of TS and NTBI were significantly higher in the high ferritin group (57.93 ± 25.07 versus 32.71 ± 18.30 [$P < .001$] and 10 ± 7.61 versus 4.17 ± 2.70 [$P = .004$], respectively). Serum levels of NTBI, ET-1, NO, TGF-β, and FGF were similar in patients with TS ≥70% versus <70% ($P > .05$). Comparison of serum levels of fibrogenic mediators and iron parameters between the 2 groups are shown in Table 2.

Comparison of Serum Iron Parameters, ET-1, NO, TGF-β, and FGF Levels between Patients with and without Chronic GVHD

There was a near significant increased incidence of chronic GVHD in the high ferritin group ($P = .057$). However, the incidence of chronic liver GVHD was similar between the high and low ferritin groups ($P = .721$). Serum values of ferritin, TS, NTBI, ET-1, NO, TGF-β, and FGF as continuous variables were similar in patients with and without chronic GVHD ($P > .05$). Serum albumin levels were significantly lower in patients with chronic GVHD (4.37 ± .38 versus 4.84 ± .36, $P = .001$). Serum ET-1 and NO levels were similar in the subgroup analysis of patients on and off immunosuppressive treatment with calcineurin inhibitors ($P > .05$).

Survival

In univariate cox regression analysis, serum levels of iron parameters, ET-1, NO, TGF-β, and FGF did not have an impact on overall survival (OS) ($P > .05$). Serum ET-1 levels were significantly higher in patients with progressive disease (76.72 ± 10.53 μmol/mL versus 91.18 ± 32.90 μmol/mL; 95% confidence interval [CI], .998 to 1000; $P = .035$). Serum levels of NO, TGF-β, and FGF did not have an impact on probability of progression-free survival (PFS) ($P > .05$).

A multivariate analysis for OS was not performed because none of the parameters showed significant impact in univariate cox regression analysis. Serum ferritin and ET-1 level were included in multivariate Cox regression analysis and showed that serum ET-1 was not an independent adverse prognostic factor for the probability of PFS ($P > .05$). The probability of OS was 74.3% (95% CI, 76.17 to 100.11) and 68.4% (95% CI, 57.36 to 94.53) in low and high ferritin groups, respectively ($P = .511$) (Figure 1A). The probability of PFS was also similar in low and high ferritin groups (56.5% [95% CI, 58.66% to 88.42%] versus 78.9% [95% CI, 64.90% to 101.30%], respectively; $P = .275$) (Figure 1B). The probability of survival was similar in patients with TS ≥70% and <70% (66.7% [95% CI, 47.95% to 101.83%] versus 73.3% [95% CI, 75.29% to 97.54%], respectively; $P = .616$). The probability of OS was also similar in patients with and without chronic GVHD ($P = .413$).

DISCUSSION

IO and ensuing free radical production through the Fenton reaction causes tissue injury in several disease and conditions, including HSCT [19]. Several previous reports showed IO before HSCT has an adverse impact on transplant outcome [11,20-22]. Serum ferritin level is considered as the surrogate marker for IO, and levels ≥1000 ng/mL are accepted as high, although lower serum ferritin thresholds are also available [13,23-25]. However, the significance of persistent IO after transplantation remains to be clarified. A retrospective series of 290 AHSCT recipients among whom 51% had ferritin levels above 1000 ng/mL, high ferritin levels showed a sustained decline, reaching normal values within 5 years. Ferritin levels at different time points (namely, 0 to 6 months, 6 to 12 months, 1 to 2 years, and 2 to 5 years) were found to be predictors of increased mortality. The adverse impact of high levels at later time points was more prominent [13]. In the current study 35.2% of recipients had serum ferritin levels ≥1000 ng/mL a year after transplantation, a frequency far less than the above-mentioned report [13]. Majhail et al. [26] reported similar results with 56 AHSCT recipients who survived ≥1 year after transplantation. The relatively small sample size and the relatively low frequency of post-transplant IO in the present cohort might explain the lack of an impact on survival. Increased serum ferritin levels showed positive correlation both with TS and NTBI as expected and supporting the role of ferritin as a marker of IO. Ferritin and TS showed a positive correlation with the impaired liver function tests at the first year after transplantation. A negative correlation between serum ferritin

Table 1
Demographic, Clinical, and Transplantation-Related Characteristics of Patients

Parameters	Value
Male/female	37/17
Age, yr (range)	25 (16-53)
Diagnosis	
Acute myeloid leukemia	21 (38.9%)
Acute lymphoblastic leukemia	5 (9.3%)
Severe aplastic anemia	13 (24.1%)
Multiple myeloma	3 (5.6%)
Lymphoma	5 (9.3%)
Chronic myelogenous leukemia	5 (9.3%)
Myelodysplastic syndrome	2 (3.7%)
Disease stages before transplantation*	
Early phase	34 (63%)
Intermediate phase	16 (29.6%)
Late phase	4 (7.4%)
Median time from diagnosis to transplantation, days (range)	237.5 (39-3018)
Donor type	
Related	53 (98.1%)
Unrelated	1 (1.8%)
$CD34^+$ cell dose, $\times 10^6$/kg	4.18 (1.7-8.76)
Preparative regimen	
Busulfan oral/Cy	16 (29.6%)
Busulfan i.v./Cy	13 (24.1%)
TBI/Cy	5 (9.3%)
Nonmyeloablative†	20 (37.2%)

Cy indicates cyclophosphamide; TBI, total body irradiation.
* Disease stage before transplantation was categorized according to the European Group for Blood and Marrow Transplantation criteria [17].
† Nonmyeloablative regimens consisted of fludarabine-based regimens, Cy, and Cy/antithymocyte globulin.

and albumin levels was found, which suggests the contribution of inflammation. The incidence of chronic GVHD was higher in the high ferritin group compared with the low ferritin group. Thus, GVHD-induced inflammation might have contributed to elevated ferritin levels.

Liver dysfunction is a common complication of HSCT. In a retrospective analysis of 121 AHSCT recipients, 52% of patients showed evidence of liver dysfunction where IO and GVHD were the most common culprits in the late post-transplant period. Almost all patients who underwent liver biopsy after day 100 showed biochemical evidence of IO and significant hepatic iron without evidence of fibrosis or cirrhosis [27]. In another series, hepatic IO was documented as the most common histological diagnosis of liver with an incidence of 75% [14]. Despite the clear association with elevated liver enzymes and IO, the association of GVHD with IO is rather controversial [15,28-30].

Serum ferritin acts as a surrogate marker of underlying inflammation as well. Although there was no correlation between serum c-reactive protein (CRP) and ferritin levels ($r = .225$ and $P = .132$), the negative correlation between the ferritin and albumin levels suggests inflammation might have contributed to elevated ferritin levels. GVHD might be partly responsible from the inflammatory milieu as a negative acute phase reactant serum albumin level was significantly lower in patients with chronic GVHD. The exact implication of high ferritin levels in the inflammatory milieu of the post-transplant setting might be complex and controversial, and more precise methods to quantify tissue iron such as magnetic resonance imaging might be required.

Serum NTBI levels at the 1-year follow-up showed significant correlation with ferritin and TS levels but did not have a correlation with liver enzymes. Previous studies have demonstrated increased cellular damage due to oxidative stress mediated by NTBI early after transplantation [31].

Serum ET-1 levels showed a negative correlation with serum ferritin and NO levels. However, levels of ET-1, NO, TGF-β, and FGF showed no correlation with liver function tests, at least at this time point, which is relatively short for hepatic fibrosis to develop. In conditions of oxidative stress, reactive oxygen species attenuate ET-1–induced NO production, causing an outweighing vasoconstriction [32]. ET-1 is released from hepatic vascular smooth muscle cells, mesenchymal cells, endothelial cells, and stellate cells in the damaged liver [33]. However, our results failed to show an association between ET-1 levels and liver enzymes, although we do not have data regarding liver histopathology. Tomis et al. [34] evaluated the levels of circulating plasma ET-1 and its role in the pathogenesis of HSCT-related renal and hepatic dysfunction in 65 HSCT recipients. Despite gradually increasing ET-1 levels from day −7 to day +28, there was no association between ET-1 levels and renal or hepatic dysfunction [34].

Despite the positive correlation between ferritin and elevated liver enzymes and the increased incidence of chronic GVHD in the high ferritin group, there was no association between iron parameters and the cytokines NO and ET-1. These results indicate that elevated serum ferritin levels either as a marker of inflammation or IO might be associated with liver dysfunction in HSCT survivors, albeit without a relation to fibrogenic cytokines. After a median follow-up of 73 months, we failed to demonstrate any effect of persistent IO on OS and PFS in AHSCT survivors of 1 year or more. This compares favorably with some previous reports [15,35]. Although IO is a common complication of HSCT, serum ferritin might not reliably predict tissue iron. The limitations of our study are the relatively limited sample size and heterogeneity of underlying disorders. IO and/or the chronic inflammatory status of the transplantation with the

Table 2
Comparison of Serum Levels of Fibrogenic Mediators and Iron Parameters

Parameters	Ferritin < 1000 (n = 34)	Ferritin ≥ 1000 (n = 19)	*P*	95% CI	
				Lower	Upper
ET-1, μmol/mL	84.46 ± 26.14	75.67 ± 7.96	.077	−.97	18.55
NO, μmol/mL	31.42 ± 27.99	30.61 ± 23.30	.910	−13.67	15.30
TGF, ng/mL	2.53 ± .67	2.66 ± .93	.590	−.63	.36
FGF, pg/mL	50.57 ± 35.93	49.29 ± 27.70	.886	−16.57	19.13
NTBI, μg/dL	4.17 ± 2.70	10.00 ± 7.61	.004	−9.58	−2.05
Ferritin, ng/mL	382.16 ± 313.18	1635.35 ± 481.10	<.001	−1505.17	−1001.21
TS, %	32.71 ± 18.30	57.93 ± 25.07	<.001	−38.62	−11.81
Serum iron, mg/dL	86.61 ± 29.47	146.52 ± 74.78	.003	−97.08	−22.73

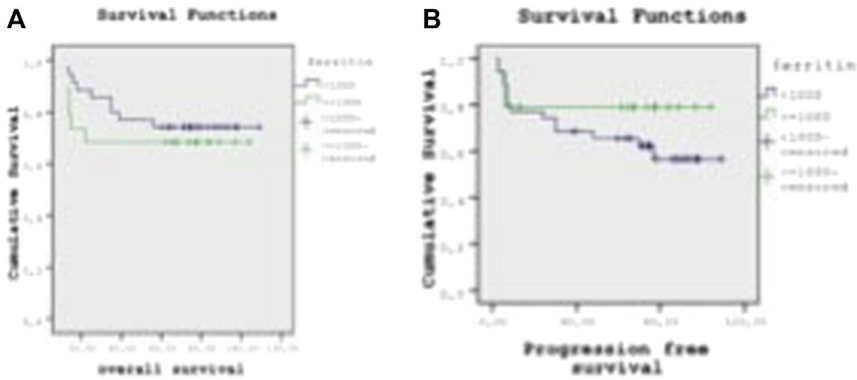

Figure 1. Probability of OS (A) and PFS (B) of patients according to ferritin levels (<1000 ng/mL versus ≥1000 ng/mL).

contribution of GVHD might be stimulating endothelium to secrete endothelin. As the negative correlation demonstrated between serum levels of ET-1 and NO, the normal interaction between the 2 might be disrupted in this context.

In conclusion, serum iron parameters have been shown to positively correlate with elevated liver enzymes in survivors of AHSCT. However, liver dysfunction showed no correlation with fibrogenic cytokines, suggesting a mild and relatively self-limited course of liver dysfunction with the current levels of ferritin and a relatively short follow-up that might be too soon for fibrosis to occur. Our results, similar to some previous reports, fail to demonstrate an impact of IO on PFS and OS. Despite the evidence of persistently elevated ferritin in allogeneic HSCT recipients, more evidence is required to elucidate the exact cause of elevated ferritin and to justify iron chelation in HSCT recipients with underlying disorders other than thalassemia.

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REFERENCES

1. Sakai M, Strasser SI, Shulman HM, et al. Severe hepatocellular injury after hematopoietic cell transplant: incidence, etiology and outcome. *Bone Marrow Transplant*. 2009;44:441-447.
2. Kida A, McDonald GB. Gastrointestinal, hepatobiliary, pancreatic, and iron-related diseases in long-term survivors of allogeneic hematopoietic cell transplantation. *Semin Hematol*. 2012;49:43-58.
3. Pullarkat V. Iron overload in patients undergoing hematopoietic stem cell transplantation. *Adv Hematol*. 2010; http://dx.doi.org/10.1155/2010/345756.
4. Deeg HJ, Spaulding E, Shulman HM. Iron overload, hematopoietic cell transplantation, and graft versus- host disease. *Leuk Lymphoma*. 2009;50:1566-1572.
5. Brissot P, Ropert M, Le Lan C, Loreal O. Non-transferrin bound iron: a key role in iron overload and iron toxicity. *Biochim Biophys Acta*. 2012;1820:403-410.
6. Emerit J, Beaumont C, Trivin F. Iron metabolism, free radicals, and oxidative injury. *Biomed Pharmacother*. 2001;55:333-339.
7. Kanda J, Kawabata H, Chao NJ. Iron overload and allogeneic hematopoietic stem-cell transplantation. *Expert Rev Hematol*. 2011;4:71-80.
8. Armand P, Kim HT, Rhodes J, et al. Iron overload in patients with acute leukemia or MDS undergoing myeloablative stem cell transplantation. *Biol Blood Marrow Transplant*. 2011;17:852-860.
9. Özyılmaz E, Aydoğdu M, Sucak G, et al. Risk factors for fungal pulmonary infections in hematopoietic stem cell transplantation recipients: the role of iron overload. *Bone Marrow Transplant*. 2010;45:1528-1533.
10. Maradei SC, Maiolino A, de Azevedo AM, et al. Serum ferritin as risk factor for sinusoidal obstruction syndrome of the liver in patients undergoing hematopoietic stem cell transplantation. *Blood*. 2009;114:1270-1275.
11. Sucak GT, Yeğin ZA, Özkurt ZN, et al. Iron overload: predictor of adverse outcome in hematopoietic stem cell transplantation. *Transplant Proc*. 2010;42:1841-1848.
12. Yeğin ZA, Paşaoğlu H, Akı SZ, et al. Pro-oxidative/antioxidative imbalance: a key indicator of adverse outcome in hematopoietic stem cell transplantation. *Int J Lab Hematol*. 2011;33:414-423.
13. Meyer SC, O'Meara A, Buser AS, et al. Prognostic impact of post-transplantation iron overload after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2013;19:440-444.
14. Sucak GT, Yeğin ZA, Özkurt ZN, et al. The role of liver biopsy in the workup of liver dysfunction late after SCT: is the role of iron overload underestimated? *Bone Marrow Transplant*. 2008;42:461-467.
15. Armand P, Sainvil MM, Kim HT, et al. Does iron overload really matter in stem cell transplantation? *Am J Hematol*. 2012;87:569-572.
16. Gosriwatana I, Loreal O, Lu S, et al. Quantification of non- transferrin-bound iron in the presence of unsaturated transferrin. *Anal Biochem*. 1999;273:212-220.
17. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease. I. Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2005;11:945-956.
18. Gratwohl A. The EBMT risk score. *Bone Marrow Transplant*. 2012;47:749-756.
19. Evens AM, Mehta J, Gordon LI. Rust and corrosion in hematopoietic stem cell transplantation: the problem of iron and oxidative stress. *Bone Marrow Transplant*. 2004;34:561-571.
20. Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in patients with thalassemia. *N Engl J Med*. 1990;322:417-421.
21. Altes A, Remacha AF, Sureda A, et al. Iron overload might increase transplant-related mortality in haematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2002;29:987-989.
22. Şıvgın S, Eser B. The management of iron overload in allogeneic hematopoietic stem cell transplant (alloHSCT) recipients: where do we stand? *Ann Hematol*. 2013;92:577-586.
23. Dadwal SS, Tegtmeier B, Liu X, et al. Impact of pretransplant serum ferritin level on risk of invasive mold infection after allogeneic hematopoietic stem cell transplantation. *Eur J Haematol*. 2015;94:235-242.
24. Tachibana T, Tanaka M, Numata A, et al. Pretransplant serum ferritin has a prognostic influence on allogeneic transplant regardless of disease risk. *Leuk Lymphoma*. 2012;53:456-461.
25. Barba P, Valcarcel D, Perez-Simon JA, et al. Impact of hyperferritinemia on the outcome of reduced-intensity conditioning allogeneic hematopoietic cell transplantation for lymphoid malignancies. *Biol Blood Marrow Transplant*. 2013;19:597-601.
26. Majhail NS, DeFor T, Lazarus HM, Burns LJ. High prevalence of iron overload in adult allogeneic hematopoietic cell transplant survivors. *Biol Blood Marrow Transplant*. 2008;14:790-794.
27. Ho GT, Parker A, MacKenzie JF, et al. Abnormal liver function tests following bone marrow transplantation: aetiology and role of liver biopsy. *Eur J Gastroenterol Hepatol*. 2004;16:157-162.
28. Mahindra A, Bolwell B, Sobecks R, et al. Elevated pretransplant ferritin is associated with a lower incidence of chronic graft-versus-host disease and inferior survival after myeloablative allogeneic haematopoietic stem cell transplantation. *Br J Haematol*. 2009;146:310-316.
29. Kataoka K, Nannya Y, Hangaishi A, et al. Influence of pretransplantation serum ferritin on nonrelapse mortality after myeloablative and non-myeloablative allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2009;15:195-204.

30. Pullarkat V, Blanchard S, Tegtmeier B, et al. Iron overload adversely affects outcome of allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant*. 2008;42:799-805.
31. Sahlstedt L, Ebeling F, von Bonsdorff L, et al. Non-transferrin-bound iron during allogeneic stem cell transplantation. *Br J Haematol*. 2001; 113:836-838.
32. Karaa A, Kamoun WS, Clemens MG. Oxidative stress disrupts nitric oxide synthase activation in liver endothelial cells. *Free Radic Biol Med*. 2005;39:1320-1331.
33. Gandhi CR, Behal RH, Harvey SA, et al. Hepatic effects of endothelin. Receptor characterization and endothelin-induced signal transduction in hepatocytes. *Biochem J*. 1992;287(Pt 3):897-904.
34. Tomis JF, Sanz-Rodriguez C, de Soria VG, et al. Plasma endothelin-1 levels after stem cell transplantation. *Bone Marrow Transplant*. 2000; 26:1199-1204.
35. Wermke M, Schmidt A, Middeke JM, et al. MRI- based liver iron content predicts for nonrelapse mortality in MDS and AML patients undergoing allogeneic stem cell transplantation. *Clin Cancer Res*. 2012;18:6460-6468.