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High Prevalence of Iron Overload in Adult Allogeneic Hematopoietic Cell Transplant Survivors

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Allogeneic hematopoietic cell transplant (HCT) recipients frequently need red blood cell transfusions, and can be at risk for developing iron overload. We studied the prevalence of iron overload in 56 adult allogeneic HCT patients who had survived for a median of 28 (range: 12-151) months from transplant. Patients were initially screened with serum ferritin, and those with serum ferritin >1000 ng/mL underwent R2 magnetic resonance imaging (MRI) of the liver, a sensitive and specific noninvasive imaging technique to measure liver iron concentration (LIC). Iron overload was defined as LIC above normal (>1.8 mg/g dry weight). Nineteen patients had serum ferritin >1000 ng/mL with a median LIC of 7.0 (range: 1.8-28.3) mg/g. The overall prevalence of iron overload was 32% (95% confidence intervals, 20%-46%). The LIC on MRI was moderately correlated with serum ferritin (ρ = .47). Iron overload is a frequent complication of allogeneic transplantation. Serum ferritin is a good screening test but does not reliably predict tissue iron overload, and estimation of LIC should be considered before initiating therapy. More studies are needed to determine the impact of iron overload on long-term morbidity and mortality in allogeneic transplant survivors.

KEY WORDS
Allogeneic stem cell transplantation • Iron overload • Late complications • Ferritin • Liver iron concentration

INTRODUCTION

Iron overload is a well-recognized cause of long-term morbidity and mortality in transfusion-dependent anemias such as thalassemia and sickle cell disease [1-3]. Allogeneic hematopoietic cell transplant (HCT) recipients frequently require red blood cell (RBC) transfusions, and pretransplant hyperferritinemia has been recently associated with an increased risk of early nonrelapse mortality (NRM) [4,5]. Transfusion-related iron overload is considered to be a complication of HCT, and recently published consensus guidelines recommend screening for iron overload in all transplant survivors [6]. However, the burden of late posttransplant iron overload and its clinical impact has not been well characterized. Therefore, we conducted a study to determine the prevalence of iron overload in long-term adult survivors of allogeneic HCT.

METHODS

Allogeneic HCT survivors followed at the University of Minnesota Blood and Marrow Transplant program were eligible for this study if they met all of the following criteria: (1) survived ≥1 year from HCT, (2) were ≥18 years of age at HCT, (3) had no contraindication for magnetic resonance imaging (MRI), and (4) had no uncontrolled infections. Patients were eligible regardless of whether they needed ongoing RBC transfusions. Patients with chronic graft-versus-host-disease (cGVHD) on a stable or tapering immunosuppressive regimen were eligible; however, those with worsening GVHD were excluded. The study protocol was approved by the institutional review board, and all participants provided informed consent prior to enrollment. Consecutive patients who had survived ≥1 year from HCT and were returning for follow-up at our center were approached for participation in the study.

Patients were initially screened for iron overload on this study using serum ferritin (normal range: 20-300 ng/mL). Those with serum ferritin of >1000 ng/mL underwent an MRI of the liver to estimate liver iron concentration (LIC, normal range: 0.17-1.8 mg/g dry tissue). The MRI measurement of LIC was based on the imaging of proton transverse relaxation rates (R2) within the liver using a 1.5T MRI machine (Siemens MAGNETOM Avanto, Malvern, PA). Comparative studies with liver biopsy and biomagnetic susceptometry using superconducting quantum interference device (SQUID) have shown this noninvasive R2 MRI technique (FerriScan®, Perth, Australia) to be highly sensitive and specific for estimating LIC [7-9]. Iron overload was defined as LIC above the normal range on MRI of the liver (>1.8 mg/g). Blood transferrin saturation (normal range: 20%-55%), bilirubin (normal range: 0.2-1.3 mg/dL), aspartate aminotransferase (AST, normal range: 0-55 U/L), alanine aminotransferase (ALT, normal range: 0-70 U/L), and alkaline phosphatase (normal range: 40-150 U/L) were also measured in all patients.

Details regarding diagnosis and transplantation were obtained from our Blood and Marrow Transplant Program database, which prospectively collects data on all HCT patients at our institution. The number of RBC transfusions administered since initial diagnosis of underlying hematologic disorder was obtained from our or referring institutions Blood Bank. Patients who had a history of but no current symptoms or signs of cGHVD and had been off immunosuppressant drug therapy for at least 6 months were classified as resolved GVHD.

The primary endpoint of this study was to estimate the prevalence of iron overload in ≥1 year survivors of allogeneic HCT. Secondary endpoints were to determine the correlation between LIC on R2 MRI and serum ferritin and transferrin saturation in patients with elevated ferritins. Patient and transplant characteristics were compared using chi-square, Fisher's exact, or Wilcoxon's rank sum test, as appropriate. Relationships between LIC and ferritin and transferrin saturation were explored using Spearman's rank correlation (ρ). All tests were 2 tailed, and a *P*-value of <.05 was considered to be statistically significant. Analysis was conducted on SPSS software (version 10.0, Chicago, IL).

RESULTS

Seventy-two allogeneic HCT recipients were approached for participation in this study. Twelve patients either refused participation (N = 5) or were not eligible for enrollment (disease relapse, 4; uncontrolled GVHD, 1; uncontrolled infection, 1; contraindication for MRI , 1). The median age at HCT and duration of follow-up since HCT were similar between patients who enrolled and did not enroll on this study. The diagnoses of patients who did not participate included non-Hodgkin lymphoma (NHL; N = 3), acute myelogenous leukemia (AML; N = 2), chronic myelogenous leukemia (CML; N = 2), Hodgkin lymphoma (HL; N = 2), multiple myeloma (MM; N = 2) and chronic lymphocytic leukemia (N = 1).

Sixty patients were enrolled in this study. Four patients did not complete study evaluations after signing consent and were excluded from further analysis. All 56 eligible patients were transfusion independent at the time of study enrollment. For the whole cohort, median age at enrollment was 51 (range: 24-69) years and patients were a median of 28 (range: 12-151) months since transplantation.

Serum ferritin of >1000 ng/mL was present in 19 (34%) patients (Table 1). Compared to patients without an elevated ferritin, patients with serum ferritin of >1000 ng/mL were more likely to have acute leukemia or myelodysplastic syndrome (MDS; 68% versus 30%, $P = .02$) and had received more RBC transfusions (median 24 versus 6, $P < .01$).

Among the 19 patients with serum ferritin >1000 ng/mL, 18 had iron overload on R2 MRI (LIC >1.8 mg/g), with a resultant overall prevalence rate of 32% (95% confidence intervals [CI], 20%-46%). LIC >5 mg/g was present in 13 patients (23% [95% CI, 13%-36%]). Among 33 patients with >2-year post-HCT follow-up, 12 (36%) had iron overload compared to 6 of 23 (26%) patients with ≤2 year follow-up ($P = .42$). Also, there was no difference in the proportion of patients with iron overload among recipients of myeloablative and reduced-intensity conditioning (RIC; 36% versus 26%, $P = .42$), related and unrelated donor grafts (30% versus 37%, $P = .59$), or active and resolved cGVHD (26% versus 44%, $P = .41$).

Six patients with LIC >5 mg/g underwent hemochromatosis mutation analysis using a buccal swab sample at the discretion of their transplant physician. Among these, 2 were found to be heterozygous for the C282Y mutation.

Although, hepatic or cardiac dysfunction was observed in 5 patients with elevated LIC, organ toxicity could be attributed to iron overload in only 2 patients. Among these 5 patients, 3 had abnormal liver function tests. Two had abnormal values that were within 2 times the upper limit of normal and could be attributed to medications. One patient with known cutaneous cGVHD on a stable immunosuppression regimen had blood alkaline phosphatase of 533 U/L but normal levels of bilirubin, AST, and ALT. Ferritin and LIC were 3502 ng/mL and 9 mg/g, respectively, and iron overload was considered to be the cause of her elevated alkaline phosphatase, which progressively decreased to 197 U/L after 4 phlebotomy sessions. Clinically evident congestive heart failure was present in 2 patients.

Table 1. *Characteristics of Patients with and without Elevated Serum Ferritin*

Characteristic	Serum Ferritin ≤1000 ng/mL (N = 37)	Serum Ferritin >1000 ng/mL (N = 19)	*P*-Value
Median age (range), years	52 (29-65)	49 (24-69)	.44
Median time since HCT (range), months	33 (12-151)	25 (12-122)	.22
Males	20 (54%)	12 (63%)	.51
Diagnosis			.02
Acute leukemia/MDS*	11 (30%)	13 (68%)	
Non-Hodgkin lymphoma	13 (35%)	3 (16%)	
Other†	13 (35%)	3 (16%)	
Conditioning regimen			.30
Myeloablative	20 (54%)	13 (68%)	
Reduced intensity	17 (46%)	6 (32%)	
Graft source			.18
Matched related donor	25 (68%)	12 (63%)	
Matched unrelated donor	1 (3%)	3 (16%)	
Umbilical cord blood	11 (30%)	4 (21%)	
Chronic graft-versus-host disease			.20
None or resolved‡	14 (38%)	4 (21%)	
Current	23 (62%)	15 (79%)	
Abnormal liver function tests§	7 (19%)	3 (16%)	.77
Median RBC transfusions (range)¶	6 (0-35)	24 (11-41)	<.01
Median transferring saturation (range), %	23 (8-45)	33 (10-76)	<.01
Median ferritin (range), ng/mL	291 (52-991)	1508 (1003-7311)	
Median LIC (range), mg/g dry weight	ND§	7.0 (1.8-28.3)	

HCT indicates hematopoietic cell transplantation; MDS, myelodysplastic syndrome; RBC, red blood cell; ND, not done; LIC, liver iron concentration.

*Includes 1 patient with acute lymphoblastic leukemia.

†Sixteen patients with "other" diagnoses included: chronic myelogenous leukemia, 8; multiple myeloma, 4; Hodgkin lymphoma, 2; chronic lymphocytic leukemia, 1; myelofibrosis, 1.

‡Resolved cGVHD was defined as discontinuation of all immunosuppressive therapy for at least 6 months.

§Defined as abnormal serum bilirubin, alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase.

¶RBC transfusions since diagnosis of underlying malignancy.

§Not done per protocol

The first, with LIC of 15.8 mg/g, presented with unexplained heart failure. An extensive workup including right and left heart catheterization revealed no discernible etiology. Although cardiac imaging to evaluate iron accumulation in the heart was not done, iron overload was considered to be a contributing factor for her cardiac dysfunction, which improved with initiation and optimization of cardiac medications and phlebotomy. The second patient had LIC of 2.8 mg/g; however, heart failure was attributed to his previously diagnosed restrictive pericarditis.

Serum ferritin was moderately correlated with LIC ($\rho = .47$, $p = .04$) (Figure 1A). Similarly, a moderate correlation was observed between transferrin saturation and LIC ($\rho = .46$, $P = .05$) (Figure 1B).

DISCUSSION

We observed a relatively high prevalence of iron overload in long-term survivors of allogeneic HCT, emphasizing the need for routine screening for iron overload in this population.

Reports of iron overload after allogeneic HCT are largely limited to case series or have only used ferritin as a marker for body iron content [10-12]. Only 1 other study has systematically evaluated iron overload in adult allogeneic HCT survivors and reported a similar high prevalence [13]. In this cross-sectional study by Rose et al. [13], 38 of 65 (58%) allogeneic HCT recipients surviving a median of 9 years since their transplant had above normal ferritin (median ferritin for whole cohort was 532 [range: 42-4203] μg/L). T2* MRI of the liver, albeit not validated for hepatic iron estimation, was done in 32 patients with elevated ferritin, and of these 31 had increased LIC (median 117 μmol/g [~6.5 mg/g]).

Serum ferritin has been shown to be a good test for screening for iron overload but a poor predictor of LIC in multiple studies in patients with thalassemia and sickle cell disease [1,14-19]. Our study demonstrates the poor predictive value of ferritin for estimating LIC in adult HCT survivors. Variability in ferritin levels because of ineffective hematopoiesis or underlying inflammation or infection could explain the lack of this association. Although a useful test for initial screening of iron overload in transplant recipients, serum ferritin is not a reliable indicator of total body iron burden. Therefore, attempts should be made to determine LIC in HCT survivors with elevated serum ferritin prior to initiating therapy for iron overload. Besides this study, other recent reports have described the use of various liver MRI techniques for estimating LIC in

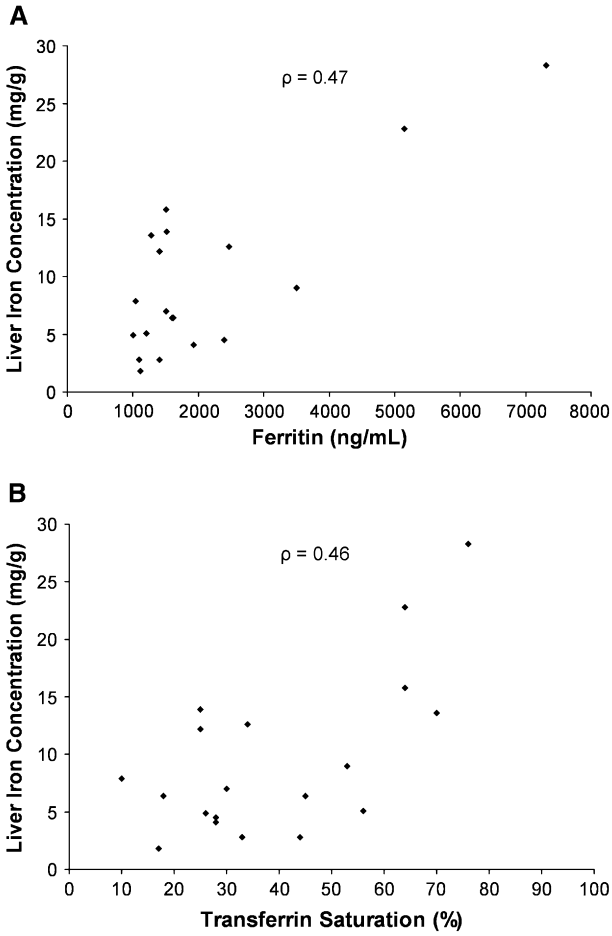

Figure 1. Relation between liver iron concentration and ferritin (A) and transferrin saturation (B) (ρ is the Spearman's rank correlation coefficient). Liver iron concentration was estimated using R2 MRI in patients with ferritin >1000 ng/mL.

HCT recipients, which allows for making a diagnosis of iron overload noninvasively [13,20]. We used the R2 MRI technique (FerriScan®) for estimating LIC in this study. This is relatively simple to setup and involves calibrating a standard 1.5T MRI machine with the proprietary FerriScan software. Images of the liver are then transmitted and analyzed centrally by the software and a report listing the LIC is generated and sent back to the investigators.

Only 2 patients with elevated LIC had clinically significant end-organ damage that could be attributed to iron overload. Because iron-induced organ toxicity typically manifests after years of developing iron overload [1], the relatively short follow-up of our cohort could explain the lack of iron-associated comorbidities. Furthermore, the natural history of transfusional iron overload in patients who become transfusion independent is not well characterized. Slow but steady decreases in LIC over time have been observed in children with thalassemia cured after allogeneic HCT, especially if they have received adequate chelation therapy and have no liver fibrosis pretransplant [21,22].

Our study has some limitations. We excluded patients with worsening GVHD. Selection bias could also have been introduced because our study cohort was a convenience sample since follow-up beyond 2 years posttransplant at our center in patients without GVHD is at the discretion of the primary transplant physician. However, a similar prevalence of iron overload was observed among patients surviving less than and longer than 2 years and among those with and without active cGVHD. Also, liver MRIs were not performed in patients with ferritin levels ≤1000 ng/mL. Iron overload might have been missed in patients with ferritin levels that were above normal but <1000 ng/mL, and as a result, we might have underestimated the true prevalence of iron overload. However, few patients with transfusion-dependent anemias and ferritin ≤1000 ng/mL have significantly elevated LIC [1,19,23].

Notwithstanding these limitations our study raises important issues about iron overload in HCT survivors. The current paradigm of management of posttransplant iron overload is largely based on experience with transfusion dependent anemias and MDS. LICs >5 to 7 mg/g are considered to herald body iron levels high enough to cause end-organ damage and necessitate the initiation of iron chelation therapy [1]. Although measurement of LIC is recommended, it is typically not done in routine clinical practice because of the invasive nature of liver biopsy and the limited availability of SQUID. Iron chelation therapy is generally initiated for persistent elevation of serum ferritin in the setting of ongoing transfusion therapy. However, the majority of HCT survivors, unlike patients with transfusion-dependent anemias, do not need long-term transfusion support after recovering from the early posttransplant period. This important difference underscores the need to ascertain body iron levels by measuring LIC, especially with the availability of noninvasive MRI techniques, before initiating iron chelation therapy or phlebotomy instead of relying on ferritin alone and highlights the need to conduct future investigations of the evolution of iron overload following transplantation.

In summary, our study demonstrates that iron overload is a relatively common complication in long-term HCT survivors. Serum ferritin is a good screening test, but estimation of LIC should be considered before initiating therapy for iron overload. More studies are needed to better define the natural history of iron overload and its impact on late morbidity and mortality in this population.

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