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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