MRI-Based Liver Iron Content Predicts for Nonrelapse Mortality in MDS and AML Patients Undergoing Allogeneic Stem Cell Transplantation

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Abstract

Purpose: Retrospective, surrogate marker–based studies have found inconsistent associations between systemic iron overload (SIO) and adverse outcome in patients undergoing allogeneic stem cell transplantation (allo-SCT). As a consequence, the impact of SIO in this context remains under debate. The aim of this study was to test whether the objective pretransplant quantification of liver-iron content (LIC) by magnetic resonance imaging (MRI) could circumvent these limitations and conclusively define the prognostic relevance of SIO.

Experimental Design: The correlation between pretransplant LIC and surrogate parameters as well as the impact of SIO on posttransplant outcome was assessed within an observational study of patients (n = 88) with either myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) undergoing allo-SCT.

Results: Ferritin levels of 1,000 ng/mL or more provided only poor specificity (31.8%) for predicting elevated LIC (>125 µmol/g) and even higher thresholds (>2,500 ng/mL) lacked an association with nonrelapse mortality (NRM). In contrast, LIC 125 µmol/g or more was a significant risk factor for NRM in uni- and multivariate analysis (HR = 2.98; P = 0.016). Multivariate Cox-regression further showed that LIC 125 µmol/g or more was associated with a decreased overall survival (HR = 2.24, P = 0.038), whereas ferritin or transfusion burden were not.

Conclusions: SIO reflected by LIC is an independent negative prognostic factor for posttransplant outcome in patients with AML and MDS undergoing allo-SCT. Therefore, MRI-based LIC, and not interference-prone serum markers such as ferritin, should be preferred for pretransplant risk stratification and patient selection in future clinical trials.

Clin Cancer Res; 18(23); 6460–8. ©2012 AACR.

Introduction

Systemic iron overload (SIO) is a frequent clinical feature in thalassemia and nontransfusion-induced iron overload conditions, such as hemochromatosis. The adverse consequences of SIO are well understood in these patients (1). Toxicity is mediated largely by redox-active, nontransferrin-bound iron that is capable of freely crossing membrane barriers (2, 3). Evidence from the clinical course of these patients indicates that iron-associated toxic effects are expected when liver-iron content (LIC) exceeds a threshold of 90 to 125 µmol/g (5–7 mg/g) dry weight, and is associated with liver fibrosis as well as cardiac and pancreatic insufficiency (4).

In patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), SIO is considered to be predominantly the result of regular blood transfusions (5). Furthermore, cytotoxic chemotherapy and ineffective hematopoiesis contribute to accumulation of iron especially in these patients. As a consequence, body iron load steadily increases during the course of the disease, resulting in SIO being present in almost all patients at the time of allogeneic stem cell transplantation (allo-SCT; ref. 6). As disease-inherent cytopenia precludes direct quantification of body iron content (e.g., with liver biopsies), most of our current knowledge on SIO in this context is based on surrogate parameters, which are not entirely iron specific. Serum ferritin levels are especially difficult to interpret in a...
systemic iron overload (SIO) occurring as a consequence of red blood cell transfusions and ineffective hematopoiesis is a frequent clinical feature in patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Retrospective, surrogate-marker–based studies have reported an association of SIO with adverse outcome after allogeneic stem cell transplantation (allo-SCT). Other studies, however, failed to reproduce these results. Therefore, the whole issue of peritransplant SIO and recommendations for pretransplant iron chelation remain under debate. In this study, we show that widely accepted clinical surrogate parameter thresholds are of limited value for the detection of SIO. Most importantly, our results indicate that SIO, if determined objectively by liver magnetic resonance imaging (MRI), has indeed a negative impact on posttransplant outcome. Therefore, liver-MRI may be better suited for risk stratification in patients with AML and MDS undergoing allo-SCT. In addition, clinical trials focusing on interventions targeting SIO in allo-SCT patients should select patients using liver-MRI.

**Translational Relevance**

Systemic iron overload (SIO) occurring as a consequence of red blood cell transfusions and ineffective hematopoiesis is a frequent clinical feature in patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Retrospective, surrogate-marker–based studies have reported an association of SIO with adverse outcome after allogeneic stem cell transplantation (allo-SCT). Other studies, however, failed to reproduce these results. Therefore, the whole issue of peritransplant SIO and recommendations for pretransplant iron chelation remain under debate. In this study, we show that widely accepted clinical surrogate parameter thresholds are of limited value for the detection of SIO. Most importantly, our results indicate that SIO, if determined objectively by liver magnetic resonance imaging (MRI), has indeed a negative impact on posttransplant outcome. Therefore, liver-MRI may be better suited for risk stratification in patients with AML and MDS undergoing allo-SCT. In addition, clinical trials focusing on interventions targeting SIO in allo-SCT patients should select patients using liver-MRI.

setting of acute and chronic inflammation, such as the peritransplantation period as ferritin concentrations are affected by circulating iron levels, inflammatory status, and cellular apoptosis (1, 7). Transfusion history should in theory be more reliable, although it does not reflect the occurrence of SIO due to inefficient hematopoiesis or chemotherapy and may also be inadequate in cases of uncontrolled bleeding. As a consequence, the prognostic and therapeutic implications of SIO in the context of allo-SCT are not well understood. Some groups have reported pretransplant SIO, reflected by ferritin levels correlated with an increased risk of relapse (8), whereas others did not observe such an association (9–11). Furthermore, while the incidence of GvHD was increased in patients with SIO in 2 studies (9, 11), 2 other groups did not see a significant impact (10, 12), whereas the data of Mahindra and colleagues (8) suggested that SIO may predict a reduced risk of GvHD. With regards to nonrelapse mortality (NRM) and overall survival (OS), the majority of published studies postulated that SIO is a predictor of an adverse outcome, although it is still under debate whether this is due to SIO or merely reflects the association between iron parameters and other adverse risk factors (8–11). Moreover, there is a considerable debate on the clinical relevant threshold for ferritin that discriminates between patients with and without SIO, with most centers using thresholds from 1,000 ng/mL (13) to 2,500 ng/mL (11).

The purpose of this prospective observational study was to investigate the influence of SIO as determined by magnetic resonance imaging (MRI) on posttransplant outcome in patients with MDS and AML undergoing allo-SCT.

**Patients, Materials, and Methods**

**Patient selection**

On the basis of the results of a recent study by our group (11), MRI-based assessment of LIC was carried out within 30 days of the allo-SCT in all subsequent patients with MDS or AML at risk for SIO. All patients provided written, informed consent, with this observational study being approved by the local ethics board. The study was conducted in accordance with the Declaration of Helsinki.

**Iron and serum parameters**

Serum iron, transferrin, ferritin, alanine-aminotransferase, aspartate-aminotransferase, bilirubin, γ-glutamyltransferase, C-reactive protein, and High Iron Fe (HFE) genotype were determined at a central laboratory using standardized, validated, and commercially available assays. All blood tests were conducted within 4 weeks before allo-SCT. Assessments of redox active iron species were not included in the original study protocol, and therefore conducted only in patients with available serum in the biobank of our institution. Preconditioning samples were examined using kits for labile plasma iron (LPI) and enhanced LPI (eLPI; both from Affex Ltd.) according to the manufacturer’s instructions. Samples were classified as negative for LPI (≤0.4 LPI-units) or positive (>0.4 LPI-units).

**Magnetic resonance imaging of the liver**

LIC was assessed using a 1.5 Tesla MRI scanner (Siemens). The MRI protocol was based on gradient recalled echo (GRE) sequences with a repetition time (TR) of 120 milliseconds, with different echo time (TE) "in phase" (4–30 milliseconds) and 2 pulse angles (20°/90°) to get T1-, proton density- (PD), and T2-images. The field of view was adapted to the abdominal diameter of the patient (30–45 cm). Slice thickness was approximately 10 mm. Usually 5 regions of interest (ROI) of 1 cm were selected for signal intensity measurement (3 for liver parenchyma and 2 for paravertebral muscle). LIC was quantified as appropriate by 2 independent investigators, according to the well-validated algorithm described by Gandon and colleagues (14) and Rose and colleagues (15).

**Statistical analyses**

The baseline patient characteristics were analyzed descriptively. Categorical variables were assessed using Fisher exact test, whereas correlations between continuous parameters were quantified by Spearman rank correlation coefficient. Receiver–operator characteristics (ROC) curves were also applied to visualize and quantify the association between continuous variables and a dichotomous outcome. The state of the underlying disease was classified as "early" in the case of MDS or AML in first complete remission (CR), whereas patients beyond first CR, with relapsed or refractory disease were classified as "advanced". Time-dependent variables were censored at time of last follow-up. Acute GvHD was defined as the occurrence of any GvHD before day +100. The cumulative incidence of acute GvHD (aGvHD)
was assessed using competing event statistics, with groups compared by Gray test. Death without GvHD was treated as a competing event. The same statistical methods were applied for analysis of NRM with relapse being the competing event. The same statistical methods were applied for analysis of NRM with relapse being the competing event. The impact of different factors on NRM was assessed further by multivariate competing events statistics in the context of an a priori fixed set of established risk factors. Namely, we included comorbidity scores, age, donor type, type of conditioning, disease stage, and time from diagnosis to transplantation. OS was determined using Kaplan–Meier estimates and the groups compared using the log-rank test. Multivariate Cox-regression analysis was applied to study the impact of different SIO parameters on OS in the context of the same a priori fixed risk factors applied in the multivariate competing events model for NRM. The proportional hazard assumption was checked by testing the hypothesis that the slope of the time-dependent variation of the regression coefficients equaled 0. All statistical analyses were conducted by applying the “R” software package (version 2.14.1 with the cmprsk package—Cran network) and SPSS statistics version 17.0 (SPSS).

Results

Patient characteristics

Over a period of 30 months, 64 AML and 24 MDS patients with a median age of 58 years were screened for LIC using MRI. The patient characteristics are shown in Table 1.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total n (%)</th>
<th>LIC &lt; 125 µmol/L n (%)</th>
<th>LIC ≥ 125 µmol/L n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>52 (59.1%)</td>
<td>26 (59.1%)</td>
<td>26 (59.1%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Female</td>
<td>26 (59.1%)</td>
<td>18 (40.9%)</td>
<td>18 (40.9%)</td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>64 (72.7%)</td>
<td>33 (75.0%)</td>
<td>31 (70.5%)</td>
<td>0.811</td>
</tr>
<tr>
<td>MDS</td>
<td>24 (27.3%)</td>
<td>11 (25.0%)</td>
<td>13 (29.5%)</td>
<td></td>
</tr>
<tr>
<td>Disease stage at allo-SCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>39 (44.3%)</td>
<td>22 (50.0%)</td>
<td>17 (38.6%)</td>
<td>0.391</td>
</tr>
<tr>
<td>Advanced</td>
<td>49 (55.7%)</td>
<td>22 (50.0%)</td>
<td>27 (61.4%)</td>
<td></td>
</tr>
<tr>
<td>HCT-CI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (0–2)</td>
<td>56 (63.6%)</td>
<td>29 (65.9%)</td>
<td>25 (56.8%)</td>
<td>0.512</td>
</tr>
<tr>
<td>High (≥3)</td>
<td>32 (36.4%)</td>
<td>15 (34.1%)</td>
<td>19 (43.2%)</td>
<td></td>
</tr>
<tr>
<td>Conditioning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIC</td>
<td>69 (78.4%)</td>
<td>32 (72.7%)</td>
<td>37 (84.1%)</td>
<td>0.300</td>
</tr>
<tr>
<td>CIC</td>
<td>19 (21.6%)</td>
<td>12 (27.3%)</td>
<td>7 (15.9%)</td>
<td></td>
</tr>
<tr>
<td>Donor type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUD</td>
<td>73 (83.0%)</td>
<td>35 (79.5%)</td>
<td>38 (86.4%)</td>
<td>0.572</td>
</tr>
<tr>
<td>MRD</td>
<td>15 (17.0%)</td>
<td>9 (20.5%)</td>
<td>6 (13.6%)</td>
<td></td>
</tr>
<tr>
<td>Graft source</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM</td>
<td>7 (8.0%)</td>
<td>0</td>
<td>7 (15.9%)</td>
<td>0.006</td>
</tr>
<tr>
<td>PBSC</td>
<td>81 (92.0%)</td>
<td>44 (100.0%)</td>
<td>37 (84.1%)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BM, bone marrow; MRD, matched related donor; MUD, matched unrelated donor; PBSC, peripheral blood stem cells.
frequently, with 44.3% having a transferrin saturation more than 80%.

Median LIC before transplantation was 125 μmol/g (ULN: 36 μmol/g; range: 25–350 μmol/g), which corresponds to the threshold for iron-related toxicity established in thalassemia and hemochromatosis (4). As liver biopsies are not feasible in most patients with AML and MDS, there is no clear LIC cut-off for clinically relevant SIO in this group. Therefore, we relied on a LIC ≥125 μmol/g as the best possible approximation for the definition of severe SIO. Post hoc analysis revealed that this a priori set threshold was very close to the threshold predicting OS with maximum accuracy (135 μmol/g; Supplementary Fig. S1). As shown in Table 1, these patients did not differ significantly from those with a lower LIC with regard to patient, disease, and treatment characteristics except for a higher percentage of bone marrow grafts transplanted in more severely iron-overloaded patients.

Correlation of liver iron content with surrogate parameters for SIO

There was a strong positive correlation between LIC and transfusion history ($r = 0.670; P < 0.001$, Fig. 1), and transfusion history proved to be an accurate predictor of LIC ≥125 μmol/g [area under the ROC: 0.789; 95% confidence interval (CI): 0.694–0.885; Supplementary Fig. S2A]. In fact, transfusion burden of at least 20 RBC, which is widely accepted as a suitable marker for iron overload (16), predicted elevated LIC (≥125 μmol/g) with a sensitivity of 79.5% and a specificity of 75.9%.

Serum ferritin levels also correlated significantly with LIC ($r = 0.640; P < 0.001$, Fig. 1). In addition, ferritin also seemed to be a valid predictor of a LIC of 125 μmol/g or more with an area under the ROC of 0.782 (95% CI: 0.684–0.881; Supplementary Fig. S2B). As expected, there was a close correlation between ferritin and C-reactive protein (CRP; $r = 0.477; P < 0.001$). Adjusting ferritin for CRP as proposed by Armand and colleagues [ref. 17; adjusted ferritin = ferritin/log10(CRP)] only marginally increased the degree of correlation with LIC ($r = 0.655; P < 0.001$) and the area under the ROC for predicting LIC of 125 μmol/g or more (0.790; 95% CI: 0.694–0.885). In most current reports on SIO, a ferritin level of 1,000 ng/mL or more is regarded as a suitable discriminator for identifying patients at risk of iron-related toxicity. This threshold in our cohort, while very sensitive (sensitivity: 90.9%), provided only poor specificity (31.8%) for predicting LIC of 125 μmol/g or more. With the goal of achieving a specificity of more...
than 80%, we chose 2,500 ng/mL as the discriminator for ferritin levels. This cut-off has been suggested by data from several groups including our own (8, 11) and resulted in a specificity of 84.1% and a sensitivity of 59.1%, which we considered more adequate for a diagnostic test. As a consequence, this threshold was used for further analyses in this study. Transferrin saturation was only associated moderately with LIC (r = 0.472; P < 0.001) and in patients with values more than 80%, there was no obvious correlation with LIC (Fig. 1).

**Redox-active iron species**

Adequately stored serum samples for assessment of redox-active iron species before conditioning for allo-SCT (LPI and eLPI) were available in 24 of 88 patients (27.3%). Nine of these patients (37.5%) were positive for LPI with a redox-active iron species before conditioning for allo-SCT with LIC (Fig. 1).

There was no significant correlation between LPI concentrations before allo-SCT and LIC (r = 0.117, P = 0.587), transfusion burden (r = −0.171, P = 0.425), or ferritin (r = −0.015, P = 0.945).

Enhanced LPI was positive in more than one-half of the screened patients (13 of 24 patients). These patients had a median eLPI of 8.43 LPI-units (range: 0.5–44.19 LPI-units).

Again, we did not observe any significant correlation between this redox active iron species and LIC (r = 0.126, P = 0.558), transfusion burden (r = −0.185, P = 0.387), or ferritin (r = 0.217, P = 0.308).

**Association of iron parameters with liver function and GvHD after allo-SCT**

Non-GvHD–associated hepatic injury after allo-SCT was limited to asymptomatic elevations of liver enzymes over the ULN. This occurred in 46 of the 88 (52.3%) patients until discharge from the transplant unit. The incidence of abnormal liver enzymes after allo-SCT was comparable between patients with a higher or lower LIC (45.5% vs. 59.1%; P = 0.286). A similar result was observed for ferritin (≥2,500 ng/mL vs. <2,500 ng/mL: 45.5% vs. 56.4%; P = 0.381) and transfusion burden (≥20 RBC vs. <20 RBC: 48.0% vs. 57.9%; P = 0.395).

Acute GvHD of any grade was diagnosed in 45 of 88 patients (51.1%), whereas clinically significant aGvHD (2–4) occurred in 25 of 88 patients (28.4%). The cumulative incidence of aGvHD 2 to 4 was not significantly different between patients with a LIC ≥125 μmol/L and those below that threshold (27.2% vs. 29.5%; P = 0.677; Supplementary Fig. S3A). The same was true for patients with a transfusion burden of more or less than 20 RBC (30.0% vs. 26.3%; P = 0.759; Supplementary Fig. S3B) or with a ferritin above or below 2,500 ng/mL (36.4% vs. 23.6%; P = 0.301; Supplementary Fig. S3C). Transferrin saturation also did not predict the occurrence of aGvHD (data not shown).

**Iron parameters and myocardial dysfunction before allo-SCT**

We evaluated the impact of iron overload on myocardial function, using 2-dimensional echocardiography data, which were available in 74 of 88 patients. Abnormal findings were reported in 30 of 74 patients (40.5%) of which 11 displayed an isolated reduced left ventricular ejection fraction, 12 had disturbed diastolic myocardial function, whereas 7 showed both systolic and diastolic dysfunction.

The proportion of patients with echocardiography abnormalities did not differ significantly between individuals with or without an elevated LIC (48.6% vs. 32.4%; P = 0.236), or with or without a ferritin level of 2,500 ng/mL or more (50.0% vs. 34.1%; P = 0.229). Interestingly, disturbed myocardial function was seen more frequently in patients transfused with 20 RBC or more (52.3% vs. 23.3%; P = 0.016).

**Iron parameters and infections before and after allo-SCT**

The overall incidence of bacterial infections after allo-SCT was similar between patients with a LIC of 125 μmol/L or more and those with a lower hepatic iron content (50.0% vs. 63.6%; P = 0.282). Comparable observations were made for ferritin and transfusion burden (ferritin above vs. below 2,500 ng/mL: 57.6% vs. 56.4%; P = 1.000 and transfusion burden less vs. ≥20 RBC: 63.6% vs. 50.0%; P = 0.282).

There was also no increased incidence of posttransplant invasive fungal infections (IFI) in patients with a LIC of at least 125 μmol/L (25.0%) as compared with those with less hepatic iron (19.9%; P = 0.429). The same applied to ferritin (above vs. below 2,500 ng/mL: 24.2% vs. 18.2%; P = 0.588) and transfusion history (above vs. below 20 RBC: 22.0% vs. 18.4%; P = 0.792). In contrast, IFI before allo-SCT were much more frequent in iron-overloaded patients no matter which parameter was applied for the definition of SIO. Indeed, the pretransplant prevalence was 31.8% in cases of LIC 125 μmol/L or more, compared with only 4.5% in cases with a lower hepatic iron content (P = 0.002). Similar significant associations were also seen between preexisting IFI and transfusion burden (>20 vs. ≤20 RBC: 28.0% vs. 5.3%; P = 0.010) as well as ferritin (above vs. below 2,500 ng/mL: 36.4 vs. 7.3%; P = 0.001).

**Impact of iron parameters on patient outcome**

Several studies have linked SIO measured by ferritin or transfusion burden to adverse outcomes after transplantation (8, 9, 11). While this may be attributable to direct iron-mediated toxicity, it may also be related to the association of SIO with other prognostically relevant factors. In fact, we found a moderate but significant correlation between the hematopoietic cell transplantation comorbidity index (HCT-CI) and ferritin (r = 0.339; P = 0.008) and transfusion history (r = 0.248; P = 0.020). In contrast, this correlation was not observed at the same extent for LIC (r = 0.193; P = 0.071). Furthermore, we observed a significant correlation of LIC (r = 0.403; P < 0.001) and transfusion burden (r = 0.356; P = 0.001) with the time from diagnosis to allo-SCT. Interestingly, no such association was seen for ferritin (r = 0.099; P = 0.356).

Neither patients with an elevated LIC, nor those with a ferritin ≥2,500 ng/mL, had an increased incidence of...
When LIC was substituted by ferritin or transfusion burden, adverse risk factor for OS (HR 2.25; P = 0.016) and identified a LIC of at least 125 μmol/g as an independent adverse risk factor for OS (HR 2.25; P = 0.036; Table 3). When LIC was substituted by ferritin or transfusion burden in this model, no significant effects on OS could be shown for these 2 parameters (Supplementary Tables S5 and S6).

Discussion

To the best of our knowledge, this is the largest study reported to date that has analyzed the impact of SIO including objective assessment of LIC by MRI in patients with MDS or AML undergoing allo-SCT. First, we found that excessive SIO (≥125 μmol/g assessed by MRI) was observed in about 50% of patients before allo-SCT. Both transfusion burden and ferritin correlated strongly with LIC, as long as adequate thresholds were applied. These results are in agreement with a recent smaller study (17). The slightly better specificity of a serum ferritin level of 1,000 ng/mL or more in their cohort (46%) probably relates to the fact that they used a lower (90 μmol/g equivalent to 5 mg/g dry weight) LIC cut-off than in our study. In both their and our

Table 2. Multivariate competing risk regression analysis for factors with potential influence on NRM

<table>
<thead>
<tr>
<th>Factor</th>
<th>HR</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>LIC (≥125 μmol/L vs. &lt;125 μmol/L)</td>
<td>2.98</td>
<td>0.016</td>
</tr>
<tr>
<td>Donor (MUD vs. MRD)</td>
<td>2.98 (0.81–11.1)</td>
<td>0.100</td>
</tr>
<tr>
<td>HCT-CI</td>
<td>1.19 (1.00–1.42)</td>
<td>0.055</td>
</tr>
<tr>
<td>Age</td>
<td>1.00 (0.96–1.04)</td>
<td>0.980</td>
</tr>
<tr>
<td>Time diagnosis to allo-SCT</td>
<td>0.98 (0.95–1.01)</td>
<td>0.130</td>
</tr>
<tr>
<td>Conditioning (CIC vs. RIC)</td>
<td>0.44 (0.12–1.55)</td>
<td>0.200</td>
</tr>
<tr>
<td>Disease stage</td>
<td>0.45 (0.188–1.08)</td>
<td>0.074</td>
</tr>
</tbody>
</table>

NOTE: HCT-CI, age, and time from diagnosis to allo-SCT were entered as continuous variables. Bold, P < 0.05; italic, P < 0.1.
cohort, a transfusion burden of 20 RBC or more provided considerably better sensitivity and specificity than certain ferritin levels for predicting an elevation of LIC. We therefore conclude that although accepted widely (16) and recommend by several clinical guidelines (18, 19), a ferritin level of 1,000 ng/ml or more is not a reliable marker for SIO in patients with AML and MDS, especially under circumstances of ongoing inflammation. According to our data, higher thresholds such as those proposed by the National Comprehensive Cancer Network (20) are better suited for identifying severely iron-overloaded patients before alloSCT. Although we do not have serial ferritin measurements for our patients, it seems reasonable to believe that they, instead of single measurements, are more robust to confounding variables, and could therefore further increase the accuracy of this parameter for the prediction of SIO. Alternatively, a transfusion burden of 20 or more RBC may be used, which is unequivocally recommended by almost all practice guidelines (18, 20, 21).

Second, and most importantly, we could show that LIC rather than ferritin or transfusion burden have a significant impact on posttransplant outcome. Although there was a certain degree of increased NRM in patients with a ferritin level of 2,500 ng/ml or more or a transfusion burden of 20 RBC or more in the early posttransplantation period, these differences were attenuated over time and did not reach statistical significance. In contrast, NRM was consistently higher over the whole posttransplant period in patients with a high LIC as compared with low LIC. Moreover, after adjusting for other well-known risk factors in a multivariate model, only a LIC of 125 μmol/l or more retained a significant impact on NRM. Multivariate Cox regression analysis also identified a LIC of 125 μmol/l or more as a significant adverse risk factor for OS, whereas ferritin or transfusion burden showed no such association. These data are in contrast to the retrospective results of other groups (8, 9, 12) and also multicenter data we have published previously (11). In all of these cohorts lacking MRI data, a significant association between transfusion dependency and/or certain ferritin thresholds and NRM as well as OS was observed. One possible reason for these conflicting results is the difference in sample size and patient characteristics between these earlier reports and the current study. This may imply that ferritin and transfusion burden correlate with NRM, but due to the dilution of their effects by multiple and variable confounding factors their impact may be weaker than that of LIC measured by MRI. Therefore, LIC may be a better discriminator for identifying patients at risk of NRM, especially in smaller patient cohorts. This idea has, however, been challenged by recent data from Armand and colleagues (22). Updating the survival data of their 45 patients first reported in 2011 (17), they were unable to show a prognostic impact of a LIC of 90 μmol/l or more (≈ 5 mg/g). In contrast, a ferritin level of 2,500 ng/ml was a significant predictor for NRM and OS in their cohort. The authors concluded that serum ferritin negatively impacts on posttransplant prognosis not because it is a marker of SIO but because it is correlated with comorbidity, inflammation, and advanced disease. Although it is hard to directly compare their cohort with ours, because their patients were younger (median age: 46 years), less severely iron-overloaded patients before alloSCT. Although we do not have serial ferritin measurements for our patients, it seems reasonable to believe that they, instead of single measurements, are more robust to confounding variables, and could therefore further increase the

Table 3. Multivariate Cox regression analysis of OS

| LIC (≥125 μmol/l vs. <125 μmol/l) | 2.32 (1.05–4.80) | 0.036 |
| Donor (MUD vs. MRD) | 2.32 (0.69–7.72) | 0.173 |
| HCT-CI | 1.03 (0.88–1.20) | 0.715 |
| Age | 1.00 (0.97–1.03) | 0.844 |
| Time diagnosis to alloSCT | 0.97 (0.94–1.00) | 0.072 |
| Conditioning (RIC vs. RIC) | 0.60 (0.22–1.67) | 0.330 |
| Disease stage (advanced vs. early) | 0.75 (0.35–1.61) | 0.754 |

NOTE: Age, HCT-CI, and time, as diagnosis were entered as continuous variables. Bold, P < 0.05; italic, P < 0.1.
by liver biopsies (14, 25), the former tends to higher LIC values when directly compared with the latter (24). Moreover, Gandon’s method if not supplemented by an additional sequence as proposed by Rose and colleagues (15) becomes inaccurate above a LIC of 300 to 350 μmol/L. Therefore, it is important to be aware of the expected LIC range of a study population at hand.

How can SIO potentially mediate NRM in the posttransplant period? Although we have observed an association between a transfusion burden of 20 RBC or more and an increased incidence of myocardial dysfunction, we do not think that cardiac iron overload plays a role in this regard, as it hardly ever occurs in allo-SCT recipients (17). Most probably this association is reflecting the severity of chronic anemia and its consequences on myocardial oxygenation. In fact, increased oxidative stress caused by reactive iron species and consecutive tissue damage has been proposed as a possible reason for the association between SIO and aGvHD by Alessandrino and colleagues (9). Indeed, we could detect redox-active iron species in more than half of the assessed patients, although the limited number of samples analyzed precluded the detection of any formal correlation between the extent of SIO and the occurrence of these molecules. We have, however, not observed an association between SIO and an increased incidence of aGvHD. This difference to Alessandrino’s results (9) may be attributable to cross-correlations of surrogate markers for SIO with confounding factors that have variable effects on the risk of aGvHD in different study populations. Alternatively, notable differences in conditioning regimens and transplant sources used between the Italian cohort (9) and ours, may indicate that SIO itself has a variable impact on aGvHD that is dependent on the respective clinical context.

Apart from causing oxidative stress, iron is also an essential cofactor for many microbial pathogens, and therefore it is intriguing to speculate that infections may be more common in iron-overloaded patients. In fact, a recent retrospective study showed posttransplant bacteremia was considerably more frequent in iron-overloaded patients (26). Although we could not confirm these data, an increased incidence of pretransplant IFI in patients with SIO was observed. A similar association was also reported by other groups using surrogate markers (27) or bone marrow iron (28) to define SIO and may relate to iron-mediated suppression of the innate immune system (29) or to the fact that fungal growth is an iron-dependent process (30). Moreover, the observed correlation between time from diagnosis to transplant and LIC as well as transfusion burden point at an increased time at risk for IFI in iron-overloaded patients.

While acknowledging the limitations of a single center observational trial, we consider that our data help to better define the adverse consequences of SIO in patients undergoing allo-SCT. We have shown that surrogate parameters are not necessarily associated with adverse posttransplant outcome and that an unbiased quantification of SIO may be necessary to detect existing differences in survival endpoints, especially in smaller patient subsets. This is of special relevance for upcoming interventional trials aimed at reducing iron-related toxicity, which should be based preferentially on quantification of SIO with MRI or other objective techniques as this clearly reduces the impact of confounding factors, such as comorbidity or inflammation.

Disclosure of Potential Conflicts of Interest
U. Platzbecker received research funding from Celgene and Novartis and is a consultant/ advisory board member of Novartis. M. Bornhäuser received research funding from Celgene, has honoraria from Speakers Bureau of MEDA, Celgene, and Novartis, and is a consultant/advisory board member of Riemser. G. Ehninger was granted travel cost compensation by Novartis. G. Weiss has honoraria from Speakers Bureau of Vifor. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Laniado, I. Theurl, M. Bornhäuser, U. Platzbecker
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Acknowledgments
The authors thank C. Theuser for management of the transplant registry and A. Liebkopf, I. Habermann, and M. Seifert for laboratory work.

Grant Support
This work was supported by a grant from the Austrian National Bank to I. Theurl (ÖNB no. 14182). The work of M. Laniado was supported by a Gerok grant of the German Research Fund (DFG). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 24, 2012; revised August 6, 2012; accepted August 25, 2012; published OnlineFirst September 18, 2012.

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