Article title: MRI-based liver iron content predicts for non-relapse mortality in MDS and AML patients undergoing allogeneic stem cell transplantation

Running title: Iron overload and prognosis in stem cell transplantation

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Statement of 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 peri-transplant SIO and recommendations for pre-transplant 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 MRI, has indeed a negative impact on post-transplant outcome. Therefore, liver-MRI may be better suited for risk stratification in AML and MDS patients undergoing allo-SCT. In addition, clinical trials focusing on interventions targeting SIO in allo-SCT patients should select patients using liver-MRI.
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 pre-transplant 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 pre-transplant LIC and surrogate parameters as well as the impact of SIO on post-transplant outcome was assessed within an observational study of patients (n=88) with either MDS or AML undergoing allo-SCT.

Results:

Ferritin levels $\geq$ 1000 ng/ml provided only poor specificity (31.8 %) for predicting elevated LIC ($\geq$ 125 µmol/g) and even higher thresholds ($\geq$ 2500 ng/ml) lacked an association with non-relapse mortality (NRM). In contrast, LIC $\geq$ 125 µmol/g was a significant risk factor for NRM in uni- and multivariate analysis (HR = 2.98; p = 0.016). Multivariate Cox-regression further demonstrated that LIC $\geq$ 125 µmol/g 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 post-transplant outcome in AML and MDS patients undergoing allo-SCT. Therefore, MRI-based LIC and not interference-prone serum markers like ferritin should be preferred for pre-transplant risk-stratification and patient selection in future clinical trials.
Introduction

Systemic iron overload (SIO) is a frequent clinical feature in thalassemia and non-transfusion-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, non-transferrin 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]. Further, 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) [6]. As disease inherent cytopenia precludes direct quantification of body iron content (e.g. with liver biopsies), most 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 setting of acute and chronic inflammation such as the peri-transplantation 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 pre-transplant SIO, reflected by ferritin levels correlated with an increased risk of relapse [8], while others did not observe such an association [9-11]. Further, while the incidence of graft versus host disease (GvHD) was increased in patients with SIO in two studies [9, 11], two other groups did not see a significant impact [10, 12], while the data of Mahindra et al. [8] suggested that SIO may predict a reduced risk of GvHD. With regards to non-relapse 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 1000 ng/ml [13] to 2500 ng/ml [11].
The purpose of this prospective observational study was to investigate the influence of SIO as determined by MRI on post-transplant outcome in MDS and AML patients undergoing allo-SCT.
Patients, materials and methods

Patient selection

Based on 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, gamma-glutamyl-transferase, C-reactive protein, and HFE genotype were determined at a central laboratory using standardized, validated and commercially available assays. All blood tests were performed within 4 weeks prior to allo-SCT. Assessments of redox active iron species were not included in the original study protocol and therefore performed only in patients with available serum in the biobank of our institution. Pre-conditioning samples were examined using kits for labile plasma iron (LPI) and enhanced LPI (eLPI) (both from Afferix Ltd. Ashkelon, Israel) 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, Erlangen, Germany). The MRI protocol was based on gradient recalled echo (GRE) sequences with a repetition time (TR) of 120 ms, with different echo time (TE) “in phase” (4-30 ms) and two pulse angles (20°/90°) in order 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 two independent investigators, according to the well validated algorithm described by Gandon [14] and Rose [15].

Statistical analyses

The baseline patient characteristics were analyzed descriptively. Categorical variables were assessed using Fisher’s exact test, while correlations between continuous parameters were quantified by Spearman’s 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 1st complete remission (CR), while patients beyond 1st 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 prior to day +100. The cumulative incidence of aGvHD was assessed using competing event statistics, with groups compared by Gray’s 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 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 zero. All statistical analyses were performed by applying the “R” software package (version 2.14.1 with the cmprsk package – Cran network) and SPSS statistics version 17.0 (SPSS, Munich, Germany).
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.

The patients were treated with both reduced and conventional intensity protocols. Reduced intensity regimens (RIC, n = 69) involved fludarabine plus TBI (≤ 800 cGy), busulfan or melphalan. Conventional intensity conditioning (CIC, n = 19) consisted of a combination of cyclophosphamide (120 mg/kg/d) and total body irradiation (TBI; 1000 - 1200 cGy) or busulfan (total dose of 12.8 mg/kg i.v.). All transplants were at least 9/10 HLA-matched. GvHD prophylaxis included calcineurin inhibitors with or without methotrexate or mycophenolat-mofetil.

Genotyping for HFE-mutations was available in 77 of the 88 patients (87.5 %). Four patients were heterozygous, while another was homozygous for the hemochromatosis-associated C282Y mutation (ferritin: 2696.8 ng/ml, transfusion burden: 17 RBC, LIC: 330 µmol/g, transferrin saturation: 83.7 %), whereas the remaining individuals had a wildtype/wildtype configuration of the HFE gene. The patient carrying the homozygous C282Y was clinically asymptomatic and had no signs of hemochromatosis-associated organ failure.

Iron status prior to allo-SCT

Every patient had received packed red blood cells (RBC) prior to transplantation, with a median of 22 transfused units (range: 1 – 127). As a result, pre-transplant ferritin was increased above the upper-limit of normal (ULN: 400 ng/ml) in all but three patients, with a median of 1928 ng/ml (range: 26 – 14179 ng/ml). Transferrin saturation was elevated frequently, with 44.3 % having a transferrin saturation above 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 AML- and MDS-patients 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 – suppl. Figure 1). As shown in Table 1, these patients did not differ significantly from those with a lower LIC with regards to patient, disease, and treatment characteristics except for a higher percentage of bone marrow grafts transplanted in more severely iron-overload patients.
Correlation of liver iron content with surrogate parameters for SIO

There was a strong positive correlation between LIC and transfusion history \((r = 0.670; \ p < 0.001, \ Figure \ 1)\), while transfusion history proved to be an accurate predictor of LIC \(\geq 125 \ \mu mol/g\) (area under the ROC: 0.789; 95% CI: 0.694 – 0.885, suppl. Figure 2A). In fact, transfusion burden of at least 20 RBC, which is widely accepted as a suitable marker for iron overload [16], predicted elevated LIC \(\geq 125 \ \mu mol/g\) with a sensitivity of 79.5 % and a specificity of 75.9 %.

Serum ferritin levels also correlated significantly with LIC \((r = 0.640; \ p < 0.001, \ Figure \ 1)\). In addition ferritin also appeared be a valid predictor of a LIC \(\geq 125 \ \mu mol/g\) with an area under the ROC of 0.782 (95% CI: 0.684 – 0.881, suppl. Figure 2B). As expected there was a close correlation between ferritin and CRP \((r = 0.477; \ p < 0.001)\). Adjusting ferritin for CRP as proposed by Armand et al. [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 \(\geq 125 \ \mu mol/g\) (0.790; 95% CI: 0.694 – 0.885). In most current reports on SIO, a ferritin \(\geq 1000 \ \text{ng/ml}\) 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 \(\geq 125 \ \mu mol/g\). With the goal of achieving a specificity above 80% we chose 2500 ng/ml as the discriminator for ferritin levels. This cutoff 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 above 80 % there was no obvious correlation with LIC (Figure 1).

Redox active iron species

Adequately stored serum samples for assessment of redox active iron species prior to 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 median of 0.95 LPI units (range: 0.71 – 3.21 LPI-units). There was no significant correlation between LPI concentrations prior to 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 upper limit of normal (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 ($\geq 2500$ ng/ml vs. $< 2500$ ng/ml: 45.5% vs. 56.4%, $p = 0.381$) and transfusion burden ($\geq 20$ RBC vs. $< 20$ RBC: 48.0% vs. 57.9%, $p = 0.395$).

Acute GvHD of any grade was diagnosed in 45 of the 88 patients (51.1%), while clinically significant aGvHD (°2-4) occurred in 25 of 88 patients (28.4%). The cumulative incidence of aGvHD °2-4 was not significantly different between patients with a LIC $\geq 125$ µmol/g and those below that threshold (27.2% vs. 29.5%; $p = 0.677$; suppl. Figure 3A). The same was true for patients with a transfusion burden of more or less than 20 RBC (30.0% vs. 26.3%; $p = 0.759$; suppl. Figure 3B) or with a ferritin above or below 2500 ng/ml (36.4% vs. 23.6%, $p = 0.301$; suppl. Figure 3C). Transferrin saturation also did not predict the occurrence of aGvHD (data not shown).

**Iron parameters and myocardial dysfunction prior to allo-SCT**

We evaluated the impact of iron overload on myocardial function, using two-dimensional echocardiography data, which were available in 74 of the 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, while 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 $\geq 2500$ ng/ml (50.0% vs. 34.1%, $p = 0.229$). Interestingly, disturbed myocardial function was seen more frequently in patients transfused with $\geq 20$ RBC (52.3% vs. 23.3%; $p = 0.016$).

**Iron parameters and infections prior to and after allo-SCT**

The overall incidence of bacterial infections after allo-SCT was similar between patients with a LIC $\geq 125$ µmol/g 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 2500 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 post-transplant invasive fungal infections (IFI) in patients with a LIC of at least 125 µmol/g (25.0 %) compared to those with less hepatic iron (15.9 %, p = 0.429). The same applied to ferritin (above vs. below 2500 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 prior to allo-SCT were much more frequent in iron-overloaded patients no matter which parameter was applied for the definition of SIO. Indeed, the pre-transplant prevalence was 31.8 % in cases of LIC ≥ 125 µmol/l, compared to 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 2500 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 ≥ 2500 ng/ml had an increased incidence of relapse, while a transfusion burden ≥ 20 RBC was even associated with a lower cumulative incidence of relapse, although this difference did not reach statistical significance (Suppl. Table S1). Patients with a high LIC had an increased cumulative incidence of NRM especially in the first 100 days after transplantation (at day 100: <125 µmol/g vs. ≥125 µmol/g: 4.7 vs. 27.3 %) with most patients succumbing to infections or GvHD (suppl. Table S2). This difference was preserved and of statistical significance in the long-term (p = 0.028; Figure 2A). A similar trend towards an increased early NRM was also seen in patients with a transfusion burden of ≥ 20 RBC (100-day-CI of NRM: 22.0 vs. 8.1 %), but was less pronounced in patients with a ferritin ≥ 2500 ng/ml (24.2 vs. 11.1 %). In contrast to LIC, these differences diminished over time and were not statistically significant (Figures 2B and C). We were also interested to determine if SIO measured by LIC, transfusion burden, or ferritin had
an impact on NRM in a multivariate competing risk regression model. We included an \textit{a-priori}
fixed set of well-known risk factors for adverse post-transplant outcome as covariates that
included donor-type, HCT-CI, age, type of conditioning, time between diagnosis and allo-
SCT, and disease stage at the time of transplantation. Using this approach, we confirmed
that a LIC $\geq$125 µmol/g was a significant predictor for NRM (HR 2.41; $p = 0.016$, Table 2). In
fact, this influence of LIC was independent of the HCT-CI and type of conditioning. In
contrast, ferritin and transfusion burden were not predictive for NRM when adjusted for the
same factors (suppl. Tables S3 and S4).

With a median follow-up of 14 months after allo-SCT (range 0.5 to 32 months) the
association of LIC with NRM translated into a trend towards a shortened OS in patients with
a pre-transplantation LIC $\geq$ 125 µmol/g ($p = 0.060$, Figure 3). Multivariable Cox-regression
analysis including the same \textit{a-priori} fixed set of covariates as the analyses for NRM,
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 demonstrated for these two parameters (Suppl. 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 prior to 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 ≥ 1000 ng/ml 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 cohort, a transfusion burden of ≥ 20 RBC 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 ≥ 1000 ng/ml is not a reliable marker for SIO in AML and MDS patients, especially under circumstances of ongoing inflammation. According to our data, higher thresholds like those proposed by the National Comprehensive Cancer Network [20] are better suited for identifying severely iron-overloaded patients prior to allo-SCT. 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 post-transplant outcome. Although there was a certain degree of increased NRM in patients with a ferritin ≥ 2500 ng/ml or a transfusion burden of ≥ 20 RBC in the early post transplantation period, these differences were attenuated over time and did not reach statistical significance. In contrast, NRM was consistently higher over the whole post-transplant period in patients with a high compared to low LIC. Moreover, after adjusting for other well known risk-factors in a multivariate model only a LIC ≥ 125 µmol/g retained a significant impact on NRM. Multivariate Cox-regression analysis also identified a LIC ≥ 125 µmol/g 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 et al. [22]. Updating, the survival data of their 45 patients first reported in 2011 [17], they were unable to demonstrate a prognostic impact of a LIC $\geq 90 \, \mu \text{mol/g} (= 5 \, \text{mg/g}).$ In contrast, a ferritin of 2500 ng/ml was a significant predictor for NRM and OS in their cohort. The authors concluded that serum ferritin negatively impacts on post-transplant 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 to ours, because their patients were younger (median age: 46 years), less severely iron-overloaded (median LIC 54 $\mu \text{mol/g} = 3 \, \text{mg/g})$ and all conditioned with CIC, it could still be reasoned that LIC in our cohort is also just another, yet more expensive, marker for comorbidity. What argues against this hypothesis is that our multivariate models were adjusted for HCT-CI scores. Moreover in our cohort significant correlations between HCT-CI scores were observed only for ferritin and transfusion burden but not for LIC. On the other hand caution is required when comparing LIC thresholds derived from cohorts analyzed by different MRI methods. Although, there is a good correlation between Gandon’s method and relaxometry based approaches [23, 24] and both methods are validated by liver biopsies [14, 25] the former tends to higher LIC values when directly compared to the latter [24]. Moreover Gandon’s method, if not supplemented by an additional sequence as proposed by Rose et al. [15], becomes inaccurate above a LIC of 300 to 350 $\mu \text{mol/g}.$ 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 post-transplant period? Although we have observed an association between a transfusion burden $\geq 20 \, \text{RBC}$ 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 et al. [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 post-transplant bacteremia was considerably more frequent in iron-overloaded patients [26]. Although we could not confirm these data, an increased incidence of pre-transplant 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.

Whilst acknowledging the limitations of a single center observational trial, we consider that our data helps to better define the adverse consequences of SIO in patients undergoing allo-SCT. We have demonstrated that surrogate parameters are not necessarily associated with adverse post-transplant 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 like comorbidity or inflammation.
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Reference List


## TABLES

### Table 1. Patient characteristics.

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<th>LIC ≥ 125 µmol/l n (percent)</th>
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<tbody>
<tr>
<td><strong>Sex</strong></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>
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</tr>
<tr>
<td><strong>Diagnosis</strong></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><strong>Disease stage at allo-SCT</strong></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><strong>HCT-CI</strong></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><strong>Conditioning</strong></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><strong>Donor type</strong></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>
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</tr>
<tr>
<td><strong>Graft source</strong></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>
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</tr>
</tbody>
</table>
Table 2. Multivariate competing risk regression analysis for factors with potential influence on NRM.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>HR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIC (≥ 125 μmol/g vs &lt; 125 μmol/g)</td>
<td>2.98 (1.23 – 7.22)</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 (advanced vs early)</td>
<td>0.45 (0.188 – 1.08)</td>
<td>0.074</td>
</tr>
</tbody>
</table>
Table 3. Multivariate Cox-regression analysis of overall survival.

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIC (≥ 125 µmol/g vs &lt; 125 µmol/g)</td>
<td>2.32 (1.05 - 4.80)</td>
<td>0.036</td>
</tr>
<tr>
<td>Donor (MUD vs MRD)</td>
<td>2.32 (0.69 - 7.72)</td>
<td>0.173</td>
</tr>
<tr>
<td>HCT-CI</td>
<td>1.03 (0.88 - 1.20)</td>
<td>0.715</td>
</tr>
<tr>
<td>Age</td>
<td>1.00 (0.97 - 1.03)</td>
<td>0.844</td>
</tr>
<tr>
<td>Time diagnosis to allo-SCT</td>
<td>0.97 (0.94 – 1.00)</td>
<td>0.072</td>
</tr>
<tr>
<td>Conditioning (CIC vs RIC)</td>
<td>0.60 (0.22 – 1.67)</td>
<td>0.330</td>
</tr>
<tr>
<td>Disease stage (advanced vs. early)</td>
<td>0.75 (0.35 – 1.61)</td>
<td>0.754</td>
</tr>
</tbody>
</table>
**LEGENDS FOR TABLES AND FIGURES**

**Table 1.** Patient characteristics. RIC – reduced intensity conditioning, CIC – conventional intensity conditioning, MUD – matched unrelated donor, MRD – matched related donor, BM – bone marrow, PBSC – peripheral blood stem cells

**Table 2.** Multivariate competing risk regression analysis for factors with potential influence on NRM. HCT-CI, age and time from diagnosis to allo-SCT were entered as continuous variables. RIC – reduced intensity conditioning, CIC – conventional intensity conditioning.

**Table 3.** Multivariate Cox-regression analysis of overall survival. Age, HCT-CI, and time since diagnosis were entered as continuous variables.

**Figure 1.** Correlation-matrix of different iron parameters. Scatter plots of all pair-wise combinations of the variables LIC, transfusion burden, ferritin, and transferrin saturation are given below the diagonal. The plots are arranged so that all plots in a row share a common Y-axis, while all plots in a column share a common X-axis. The names of the X- and Y-axes are shown in the gray boxes in bold letters at the top of the column and right of the row, respectively. The respective Spearman’s rank correlation coefficients are given as overlay in each scatter plot. All the correlations were statistically significant: ** - p < 0.001, * - p < 0.01.

**Figure 2.** Cumulative incidence of NRM. Cumulative incidence with respect to LIC is given in A), while transfusion burden and ferritin are used as grouping variables in B) and C). Competing events statistics were used to calculate the incidences and significance.

**Figure 3.** Kaplan-Meier plot of the probability of overall survival with respect to LIC. Significance was calculated using the log rank test.
Figure 1)
Figure 3)
Clinical Cancer Research

MRI-based liver iron content predicts for non-relapse mortality in MDS and AML patients undergoing allogeneic stem cell transplantation

Martin Wermke, Anne Schmidt, Jan Moritz Middeke, et al.

Clin Cancer Res  Published OnlineFirst September 18, 2012.

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