High transferrin saturation predicts inferior clinical outcomes in patients with myelodysplastic syndromes

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Abstract

Iron overload (IO) reflected by elevated ferritin is associated with increased mortality in myelodysplastic syndromes (MDS), however, ferritin is an imperfect metric. Elevated labile plasma iron correlates with clinical outcomes and transferrin saturation (TSAT) >80%, but is not readily measurable. The trajectory of TSAT, and its association with clinical outcomes remain undefined. Canadian MDS registry patients were evaluated. Mean TSAT, mean ferritin and transfusion dose density (TDD) were determined. Survival was evaluated by TSAT and ferritin (<50%, 50-80%, >80%), (≤500 µg/L, 501-800 µg/L, >800 µg/L). In 718 patients, median age was 74 years; 12%, 31%, 29%, 15% and 13% were IPSS-R very low, low, intermediate, high and very high. TSAT and ferritin were moderately correlated (r=0.63, *P*<0.0001). TSAT increased over time in transfusion-dependent patients (*P*=0.006). Higher TSAT and ferritin were associated with inferior 5-year overall (OS), progression-free (PFS), and leukemia-free survival (LFS) (*P*≤0.008) and higher TDD with inferior 5-year OS. TSAT >80% trended with inferior cardiac death-free survival (*P*=0.053). In univariate analysis, age, IPSS-R, blast percentage by Eastern Cooperative Oncology Group Performance Status, frailty, Charlson Comorbidity Index, iron chelation (Y/N), TDD, TSAT and ferritin were significantly associated with inferior OS. By multivariable analysis, TSAT >80% (*P*=0.007) remained significant for OS (R^2 30.3%). In MDS, TSAT >80% and ferritin >800 µg/L portended inferior OS, PFS and LFS. TSAT may indicate the presence of oxidative stress, and is readily measurable in a clinical setting. The relationship between TSAT and cardiac death-free survival warrants further study.

Introduction

Myelodysplastic syndromes (MDS) are clonal disorders of ineffective erythropoiesis leading to peripheral blood cytopenias and an increased risk of progression to acute myeloid leukemia (AML). Red blood cell (RBC) transfusion remains a cornerstone of supportive care. RBC transfusion dependence is an independent prognostic factor associated with inferior overall survival (OS) in MDS,[1-3] and higher transfusion dose density (TDD) is associated with inferior progression-free survival (PFS).[4] However, the relative contributions of underlying disease biology *versus* transfusion-driven iron toxicity are areas of active investigation. Compared to patients with transfusion-dependent (TD) hemoglobinopathies, MDS patients have shorter life expectancies and thus may be less likely to manifest organ damage from iron overload (IO).[5] Rather, it has been hypothesized that IO may lead to oxidative stress[6] which further impairs hematopoiesis,[7] accelerates mutagenesis and results in progressive marrow failure leading to disease progression and death.[4]

Serum ferritin level above 1,000 µg/L is associated with increased mortality in MDS.[1] However, underlying inflammation confounds interpretation of ferritin, rendering it

an imperfect metric of true IO. Elevated levels of oxidatively damaging non-transferrin bound iron (NTBI) and labile plasma iron (LPI) correlate with transferrin saturation (TSAT) >70% and >80% respectively,[5,8] but NTBI and LPI assays are not readily available and lack international standardization. The trajectory of TSAT in MDS patients, and its association with clinical outcomes has not been defined.

Using the Canadian MDS registry, we aimed to describe trends in TSAT, and to determine if elevated TSAT correlates with ferritin level, and/or predicts for clinical outcomes in MDS.

Methods

Patients

This was a prospective observational study using the Canadian National MDS registry, which captures detailed disease and patient-related characteristics of MDS patients from 15 Canadian centers. Registry details have been described previously.[9] Briefly, the registry enrolled patients with MDS, chronic myelomonocytic leukemia (CMML), and low blast count AML with MDS-related changes, within 1 year of diagnosis. Patients were evaluated every 3-6 months until death, loss to follow-up, or withdrawal of consent. We conducted this study with over 11.5 years of prospectively-collected patient data. All patients provided consent to participate, and the registry was approved by the research ethics boards of participating institutions.

Temporal trends in iron parameters

Trends in iron parameters were evaluated among time-varying transfusion dependent (TD) and transfusion-independent (TI) patients, where transfusion-dependence was determined at 6-month intervals. TSAT was calculated as serum iron/total iron binding capacity (TIBC). Mean values of ferritin and TSAT were calculated every 6 months, including values from 3 months prior to and following that visit. If a patient had multiple measurements during each time window, the average was calculated. Natural logarithm transformation was applied for normalizing the distribution of ferritin data by the Shapiro-Wilk normality test. Linear regression was used to evaluate a correlation between ferritin and TSAT. Linear mixed model analysis with repeated measures per subject was used to evaluate changes in ferritin and TSAT over time from enrollment up to 42 months.

Transfusion density

Transfusion dependence was defined as a minimum of one unit per 8 weeks for a duration lasting ≥16 weeks.[10] Among TD patients, time-varying TDD was defined as the total number of units transfused per month since the date of first transfusion. Median TDD for all TD patients was calculated at landmark year 1. Patients who died before 1 year were excluded from this analysis. “Low-TDD” and “High-TDD” were defined as below or above the median. For comparison, a parallel survival analysis was done in which TDD was defined by the revised International Working Group (IWG) criteria of low (≥0.75-<2 units/month) and high (≥2 units/month) transfusion burden.[11] Each patient was categorized as TI, low-TDD or high-TDD at sequential 6-month intervals. In order to compare characteristics among the three transfusion groups, a Kruskal-Wallis non-parametric test for continuous variables, and a Fisher exact test or Chi-squared test was applied for categorical variables. A *P* value of <0.05 was considered statistically significant.

Clinical outcomes

Progression was defined as death or progression to AML. Those with AML at diagnosis by contemporary definitions (20-30% blasts) were excluded from progression-free (PFS) and leukemia-free survival (LFS) analyses. OS, PFS, LFS, cardiac death-free survival (CDFS) and cumulative incidence of death from infection were each evaluated by TDD (TI, low-TDD and high-TDD), TSAT category (<50%, 50-80%, >80%), and ferritin category (≤500 µg/L, 501-800 µg/L, >800 µg/L). A patient’s TSAT and ferritin were calculated using the mean of all values from enrolment onwards, provided there had been ≥6 months of follow-up. OS analyses used only those patients surviving ≥1 year, whereas PFS and LFS were calculated in all patients from enrolment. Log-rank tests were used to detect differences in OS, PFS, LFS and CDFS, and Gray’s test was used to detect differences in cumulative incidence of infectious death. All analyses were repeated among lower risk (Revised International Prognostic Scoring System [IPSS-R] very low, low and intermediate) and higher risk (IPSS-R high or very high) patients separately. Univariate and multivariable (MVA) Cox proportional hazards analyses identified variables with a significant impact on OS.

Results

Patient characteristics

Seven hundred and eighteen patients were included. Patient characteristics are listed in Table 1. Median age was 74 years. Collectively, 67% were IPSS-R very low, low or intermediate risk. With a median follow-up of 2.1 years, actuarial OS was 2.7 years (95% confidence interval [CI]: 2.4-3.2). Seventeen percent developed AML and 61% have died. Fifty-six percent had at least one infection, and 7% had a cardiac event leading to hospital admission or death. Among 363 patients for whom cause of death was known, AML, MDS progression, infection, cardiac events,

Table 1. Characteristics of 718 myelodysplastic syndrome patients from the Canadian MDS Registry.

Characteristics	TOTAL (N=718)
Median age in years (IQR)	74 (67-80)
Median time from diagnosis to enrolment in months (IQR)	3.7 (1.2-13.9)
Male sex, N (%)	453 (63.3)
ECOG performance status, N (%)	
0	299 (42.1)
1	341 (48.0)
2	62 (8.7)
3	8 (1.1)
4	1 (0.1)
Diagnosis type, N (%)	
Primary	651 (91.2)
Secondary	63 (8.8)
WHO subtype, N (%)	
MDS-MLD	203 (28.3)
MDS-MPN, CMML-0, CMML-1 OR CMML2	87 (12.1)
MDS-EB1	80 (11.1)
MDS-EB2	81 (11.3)
MDS-RS-SLD	63 (8.8)
MDS-SLD	50 (7.0)
Secondary AML, AML (previously RAEBT), T-AML	35 (4.9)
Isolated del5q	34 (4.7)
MDS-RS-MLD	35 (4.9)
MDS-MPN-RS-T	9 (1.3)
MDS-U	36 (5.0)
Unknown	5 (0.7)
Transfusion, N (%)	
TI	500 (69.6)
TD	218 (30.4)
IPSS-R categories, N (%)	
Very low	81 (11.3)
Low	210 (29.2)
Int	192 (26.7)
High	102 (14.2)
Very high	84 (11.7)
Unknown	49 (6.8)
Median blasts, n=714, % (IQR)	3.0 (1.5-6.0)
Ring sideroblasts ≥15, N (%)	197 (27.4)
Iron chelation treatment (yes) (n=718), N (%)	95 (13.2)
Among survivors (n=277), N (%)	37 (13.4)
Among non-survivors (n=383), N (%)	58 (13.2)
Median duration iron chelation in months (IQR) (n=95)	15.4 (3.7-37.3)
Median ferritin at enrolment, mg/L (IQR) (n=718)	443.0 (182.0-1,004.0)
Ferritin categories at enrolment, μg/L (%)	
≤500	383 (53.3)
501-800	112 (15.6)
>800	223 (31.1)
Median TSAT at enrolment (n=718), N in % (IQR)	43 (28-65)
Median TSAT over time in patients ever on ict, N (range)	64 (45-91)
Median TSAT over time in patients never on ict, N (range)	41 (27-64)
Comordbidities at enrollent, total n=718, N (%)	
Diabetes	107 (14.9)
Myocardial infarction	40 (7.0)
Congestive heart failure	22 (3.9)
Cerebrovasular disease	41 (7.2)
Valvular heart disease	29 (5.1)
Dementia	11 (1.9)
Chronic pulmonary disease	66 (9.2)
Moderate-severe renal disease	25 (4.4)
Lymphoma or other solid tumour	94 (13.1)
Deep vein thrombosis/pulmonary embolism	17 (3.0)

MDS: myelodysplastic syndrome; IQR: interquartile range; ECOG: Eastern Cooperative Oncology Group; WHO: World Health Organization; INT: intermediate; TI: transfusion-independent; TD: transfusion-dependent; IPSS-R: International Prognostic Scoring System- Revised; TSAT: transferrin saturation; ICT: iron chelation therapy.

and bleeding accounted for 26%, 20%, 19%, 9.6% and 6.3% of causes, respectively. Among those with infection, pulmonary was the most common source.

Transfusion dose density

Among 500 patients who were TI at baseline, 107 (14.9%) became TD, at a median time to onset of 3.4 months. Among 545 patients who survived ≥1 year, the median TDD at landmark year 1 was 2.74 units/month (interquartile range [IQR], 1.47-4.11). Thus, low-TDD was defined as ≥1 unit/8 weeks but <2.7 units/month, and high-TDD as ≥2.7 units/month. Transfusion density was significantly associated with IPSS-R category (P<0.0001) but not with age, sex, MDS subtype or cytogenetic risk group (*Online Supplementary Table S1*). The proportion that were TD remained stable over time in all patients (P=0.53) and in the subset with an IPSS-R score of ≤4.5 (P=0.73) but increased from enrollment up to 42 months in those with a IPSS-R score >4.5 (P=0.16) (data not shown).

Correlation of transferrin saturation and ferritin

Ferritin and TSAT were only moderately correlated (r=0.63, P<0.0001) across all patients regardless of transfusion dependence. Moderate correlations were also found among time-varying TD (r=0.61) and TI patients (r=0.56). Similarly, mean TSAT increased with incremental ferritin category (Figure 1A). Among 1,657 ferritin levels ≤1,000 µg/L, 6%, 21% and 74% were associated with TSAT >80%, 50-80% and <50%. Conversely, among 680 ferritin levels ≥1,000 µg/L, 39%, 35% and 26% were associated with TSAT >80%, 50-80% and <50% (Figure 1B).

Temporal trends in transferrin saturation and ferritin

Ferritin increased from enrolment to 42 months in all patients (P<0.0001), time-varying TD (P<0.0001) and TI patients (P<0.0001). Conversely, TSAT remained stable over time in all patients (P=0.094) and time-varying TI patients (P=0.98) but increased in TD patients (P=0.006) (Figure 2).

Survival outcomes among transferrin saturation, ferritin and transfusion density groups

Higher TDD at landmark year 1 was associated with inferior OS (Figure 3), with a 5-year OS of 52%, 44% and 25% among those who were TI, low-TDD and high-TDD, respectively (P<0.0001). OS curves were similar using revised IWG definitions of low and high transfusion burden, with the exception of low-TDD patients where 5-year OS was 33% (95% CI: 22-50) (*Online Supplementary Figure S1*). When lower and higher risk patients were analyzed separately, higher TDD was associated with inferior OS in lower but not higher risk patients (*Online Supplementary Figure S2*).

Higher mean TSAT and ferritin were each associated with inferior OS, PFS and LFS (Figure 4). Five-year OS was 46%, 43% and 17% among patients with a mean TSAT <50%, 50-80% and >80% respectively (P=0.001), and was 49%, 47% and 32% among patients with ferritin ≤500 µg/L, 501-800 µg/L and >800 µg/L, respectively (P=0.003). Among patients with a mean ferritin >800 µg/L, stratifying by TSAT ≤80% *versus* >80% further discriminated differences in 5-year OS (P=0.014), and this was also seen when lower (P=0.030) and higher risk (P=0.046) patients were analyzed separately. Higher TSAT was associated with inferior OS in lower risk patients (P=0.003), with a trend toward significance in higher risk patients (P=0.16) (*Online Supplementary Figure S3*). Conversely, an association with inferior PFS and LFS was seen only in higher risk patients (P=0.005 and P=0.031, respectively) (*Online Supplementary Figure S4*). Ferritin was only associated with inferior OS, PFS and LFS among lower risk patients (P=0.003, P=0.024,

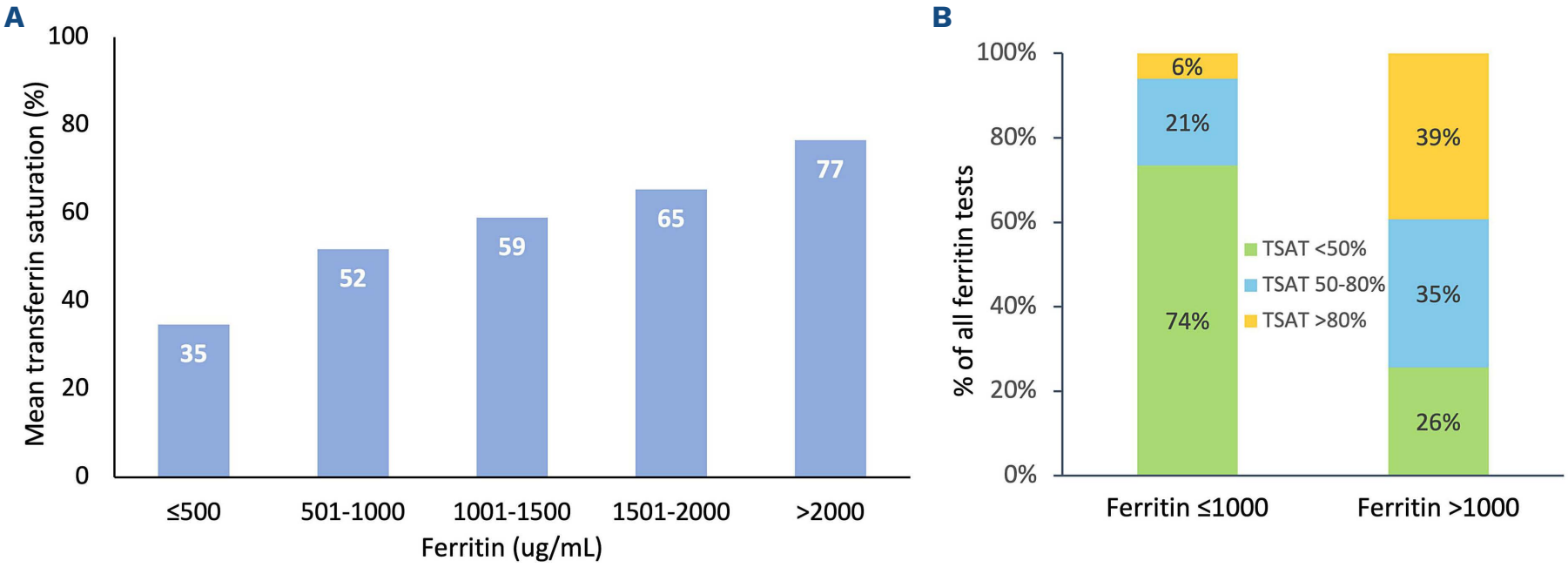

Figure 1. Correlations between transferrin saturation and ferritin across all patients regardless of transfusion status, collected from enrolment up to 42 months. (A) Mean transferrin saturation (TSAT) increased with incremental ferritin category (B) TSAT stratified by by ferritin category. Only 39% of all ferritins >1,000 µg/L were associated with a TSAT of >80%.

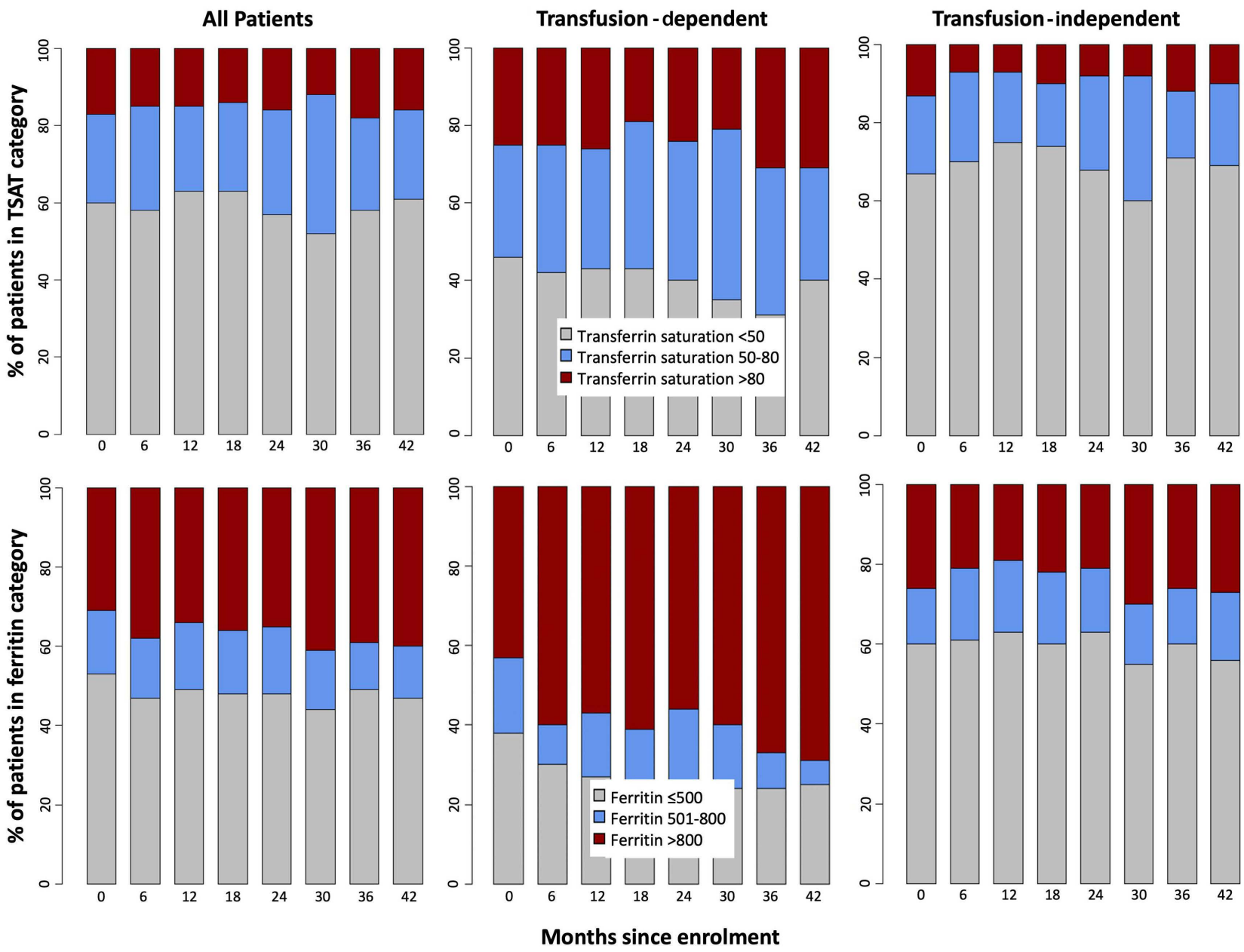

Figure 2. Temporal trends in transferrin saturation and ferritin among 718 patients with myelodysplastic syndromes. The stacked bar plots are showing the proportions of patients with ferritin ≤500, 501-800 and >800µg/L, and the proportions of patients with transferrin saturation (TSAT) <50%, 50-80%, and >80% at 6-month intervals from enrolment up to 42 months in all patients, in time-varying transfusion-dependent and in time-varying transfusion-independent patients, where transfusion dependence was determined at sequential 6-month intervals.

P=0.018 respectively).

Because iron chelation therapy (ICT) can affect measurement of TSAT, we assessed the impact of TSAT on survival according to chelation status (ever *vs.* never). 13.4% of patients received ICT. The median duration of ICT was 15.4 months (IQR, 3.7-37.3). Higher mean TSAT maintained a statistically significant association with inferior OS (*P*=0.017), although ICT attenuated this effect both in patients with mean TSAT 50-80% and >80% (Figure 5). A similar attenuating effect of ICT was seen on PFS and LFS (*Online Supplementary Figure S5*).

Cardiac events resulting in death occurred in 52 patients. Mean TSAT >80% showed a borderline association with inferior CDFS (*P*=0.053). When looking only at patients who were already TD at enrolment, mean TSAT (*P*=0.001) but not ferritin (*P*=0.52) was significantly associated with inferior CDFS (Figure 6A and B).

Seventy patients died from infection at a median of 2 years from enrolment. Ferritin >800 µg/L was associated with cumulative incidence of infectious death (*P*=0.021), however TSAT category was not (*Online Supplementary Figure S6*).

Impact of covariates

By univariate analysis, age, IPSS-R, blast percentage, Eastern Cooperative Oncology Group Performance Status (ECOG), frailty score, Charlson Comorbidity Index, not receiving ICT, TDD, TSAT and ferritin categories were significantly associated with inferior OS (*Online Supplementary Table S2*). In two MVA models with nearly identical R^2 values, TSAT emerged as an independent predictor of OS after adjusting for significant covariates (age, IPSS-R, frailty, Charlson Comorbidity Index and receipt of ICT), however either ferritin or TDD fell out of each model. We then tested the Spearman correlations between each of these three covariates as continuous variables. Ferritin was the most redundant variable, again correlating moderately with TSAT (r=0.64, *P*<0.0001) and TDD (r=0.46,

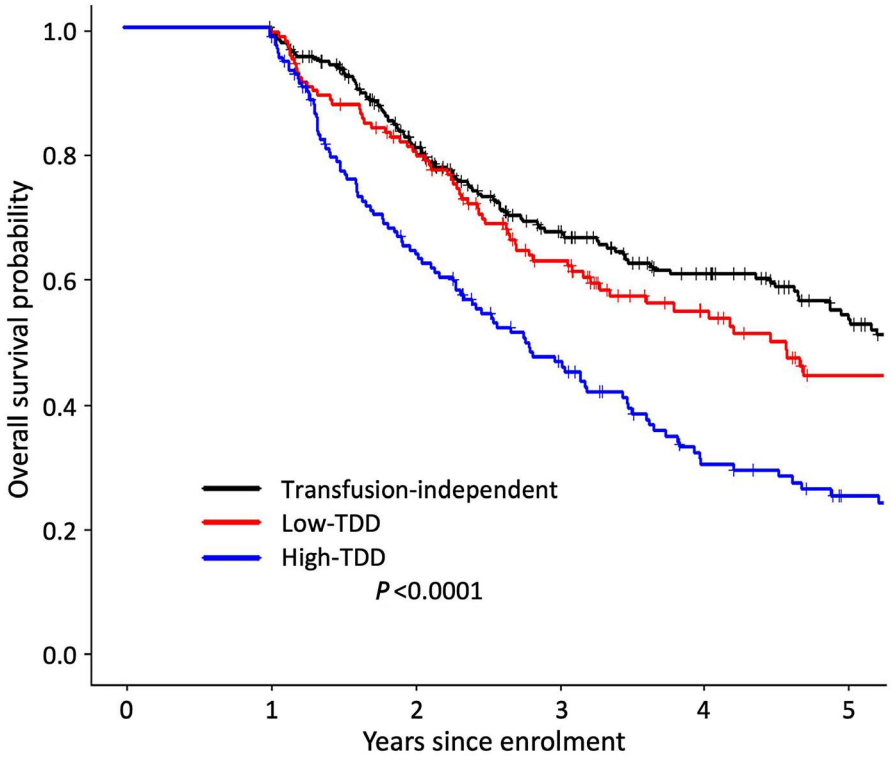

Figure 3. Overall survival of myelodysplastic syndrome patients. Higher transfusion dose density (TDD) at landmark year 1 is associated with inferior overall survival among all myelodysplastic syndrome patients.

P<0.0001), while TSAT correlated weakly with TDD (r=0.37, *P*<0.0001).

Discussion

In this retrospective analysis of a large prospectively collected cohort of MDS patients, higher mean TSAT and mean ferritin were each associated with inferior OS, PFS and LFS. The impact of IO on clinical outcomes was not restricted to lower risk patients, as demonstrated by the impact of TSAT on PFS and LFS in higher risk patients. However, TSAT correlated only moderately with ferritin, and unlike ferritin, remained stable over time in TI patients, suggesting that TSAT may be a more appropriate marker of total body iron in MDS patients.

The importance of identifying clinically significant biomarkers of iron toxicity in MDS is well-accepted. Data from the EUMDS registry showed that LPI levels correlate strongly with TSAT, with a threshold effect seen above a TSAT of 80%, and are associated with inferior survival in lower risk MDS.[5] A weaker correlation[5] or no correlation[8] was found between ferritin and LPI. TSAT is a much more readily available, standardized and cost-effective iron parameter in the clinical setting than LPI.

In comparing temporal trends of iron parameters, TSAT increased over time only in TD patients. This is consistent with data from the EUMDS registry and suggests that elevated TSAT is specific for transfusional IO.[5] Conversely, ferritin increased over time in all subgroups including TI patients, emphasizing its role as a biomarker of inflammatory processes beyond transfusional IO. Ineffective erythropoiesis (IE) may contribute to the elevated ferritin in TI patients, particularly those with ring sideroblasts (MDS-RS).[5] However, the stability of TSAT among TI patients argues against IE as the main driver of ferritin (as a surrogate of iron overload) in TI patients.

The development of transfusion dependence in MDS is associated with inferior OS,[3] and ICT is associated with improved OS[12] after adjusting for IPSS-R, a finding that we recapitulated in our MVA. In fact, the World Health Organization-based prognostic scoring system (WPSS) incorporated transfusion dependence as an independent prognostic factor for OS and LFS.[10] Higher TDD is associated with poor OS,[1,2] PFS[4] and LFS1,[13] albeit in heterogeneous MDS populations; the association of TDD with PFS was recently shown among low risk MDS patients.[4] We expand this to include an impact on OS in lower risk MDS. We also provide clinically significant TDD thresholds that predict for inferior OS.

The median TDD in this study, 2.7 units/month, was higher than that reported in the European MDS Registry (EUMDS) registry (0.87 units/month)[4] likely due to the inclusion of higher risk patients in our cohort, and a TDD definition in the EUMDS registry that divided the number of transfused units over a longer time interval. When we repeated our analysis using the 2018 IWG criteria[11] of low (≥0.75-<2 units/month) and high (≥2 units/month) TDD, we found that OS was also inferior in low-TDD patients. While the optimal definition of low-TDD requires further clarification, it is clear that any amount of transfusion dependence associates with inferior outcomes.

We demonstrated that higher TSAT and ferritin are associated with inferior OS, PFS and LFS. Higher TSAT was associated with reduced survival, particularly among lower-risk patients but with a compelling trend toward significance among higher-risk patients. These findings contrast with data from both the EUMDS and Piedmont registries. The Piedmont data used TSAT levels at baseline only.[14] The EUMDS authors found no adverse impact of

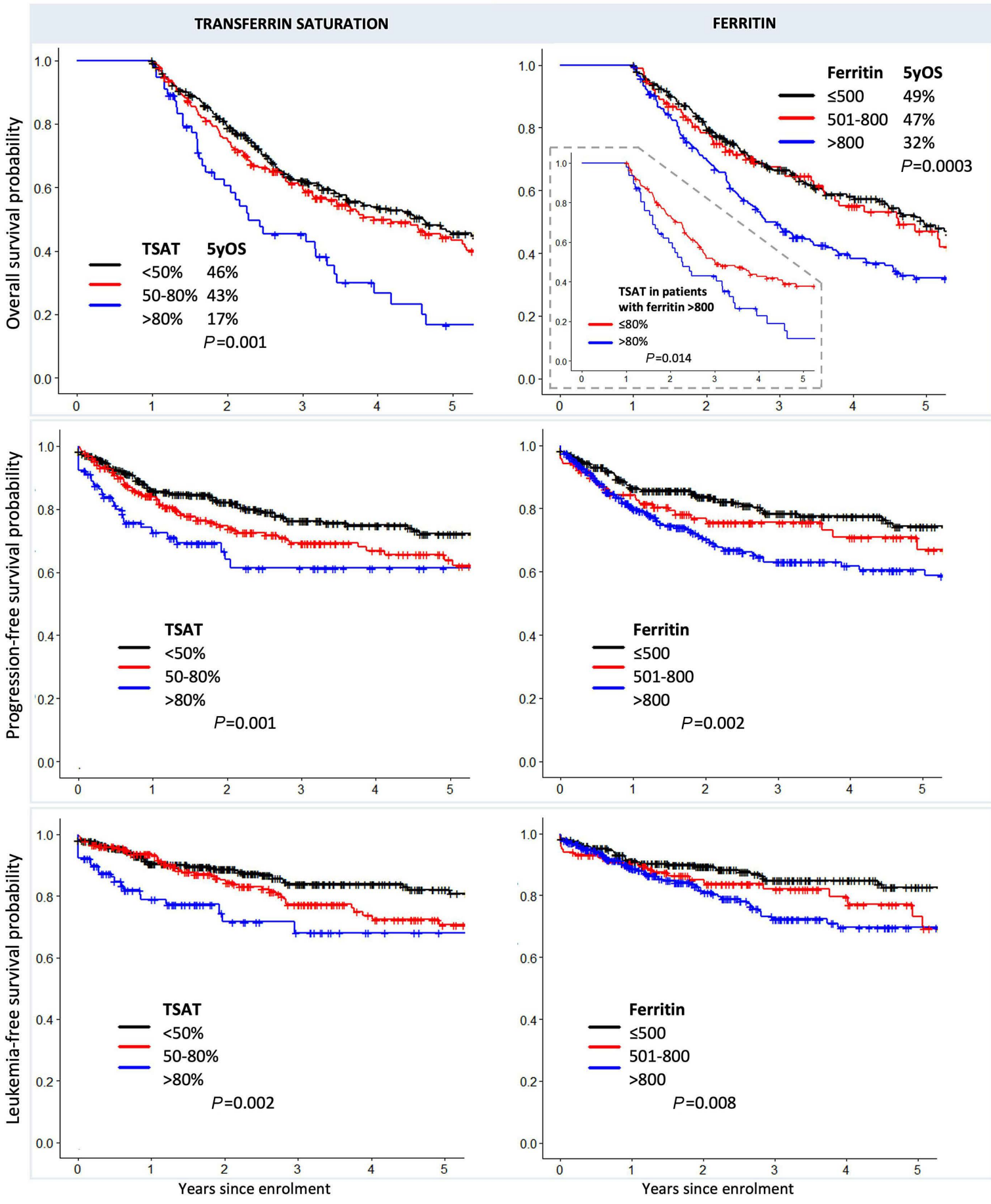

Figure 4. Higher mean transferrin saturation and ferritin are associated with inferior overall survival, progression-free survival and leukemia-free survival. Among those with a mean ferritin >800 µg/L, stratifying by transferrin saturation (TSAT) category further discriminated differences in overall survival (OS) (*P*=0.014) (dotted inlay). Total numbers of patients at time zero in the black, red and blue groups, respectively, were as follows: 302, 188 and 55 for OS analyses by TSAT category; 248, 100 and 197 for OS by ferritin category; 391, 237 and 90 for progression-free survival and leukemia-free survival by TSAT category; 309, 119, and 290 for progression-free survival and leukemia-free survival by ferritin category.

TSAT on PFS after adjusting for age, IPSS-R, receipt of erythropoietin-stimulating agents, and ring sideroblast status. They concluded that TSAT should be used as a screening test to identify patients at risk of elevated LPI.[5] The EUMDS cohort was small (n=100), restricted to patients with lower risk MDS, and defined TSAT as a time-varying covariate (<80% or ≥80%) at each six monthly visit. In our cohort of 718 patients, those with a mean TSAT ≥80% would have had a longer duration of TSAT elevation, and may therefore have been more likely to manifest clini-

cal sequelae of IO.

Previous work from Malcovati *et al*. demonstrated that ferritin ≥1,000 µg/L adversely impacts OS after adjusting for TDD, with a hazard ratio of 1.36 for every 500 µg/L above 1,000 µg/L.[1] This effect was not statistically significant among higher risk MDS subtypes. Data from the Piedmont registry similarly showed inferior OS with a baseline ferritin >800 µg/L among lower but not higher risk patients.[14] Our ferritin data are in agreement with these. Malcovati *et al*. posited that only patients with lower risk MDS subtypes survived long enough to manifest the sequelae of tissue IO. The compelling association between TSAT >80% and inferior OS in our higher risk patients argues against this. Furthermore, that ferritin and TDD were interchangeable in our MVA after adjusting for TSAT suggests that both metrics are influenced by the degree of underlying bone marrow failure. Thus, while ferritin appears to be an adequate marker of IO in lower risk disease, it may be confounded by worsening bone marrow failure, infections and inflammation in higher risk patients. That higher TSAT also associates with inferior PFS and LFS, particularly in higher risk patients, supports the hypothesis that LPI may drive mutagenesis, progressive bone marrow failure and progression to leukemia. Preclinical and translational evidence of the impact of IO on DNA mutation rates, error-prone DNA repair, loss of hematopoietic stem cell quiescence and bone marrow failure is extensive, and has been reviewed elsewhere.[15] Higher risk patients may be particularly vulnerable,[16] possibly due to a higher burden of clonal complexity and/or chromosomal instability compared to lower risk patients.

Cardiac disease is a leading cause of death in MDS.[17,18] The rate of cardiac death in this study was remarkably similar to that reported elsewhere.[18] Clonal hematopoiesis may contribute to accelerated cardiovascular disease via increased inflammatory markers.[19] IO may also contribute; cardiomyocytes rely heavily on mitochochondrial activity, which is particularly susceptible to iron-driven oxidative damage.[15] Transfused chelated MDS patients have a delayed time to cardiac event[20] and a trend toward fewer cardiac conditions compared to their non-chelated counterparts.[21] In the randomized placebo-controlled TELESTO trial, worsening cardiac function and cardiac death were less common with ICT, albeit with small sample sizes.[22] We found that while ferritin >800 µg/L did not predict CDFS (*P*=0.90), TSAT >80% showed a trend towards significance (*P*=0.053). Interestingly, TSAT and CDFS were associated in patients who were TD at baseline, suggesting that a prolonged duration of transfusions is required to accumulate clinically meaningful cardiac iron and/or oxidative damage to cardiomyocytes. Alternatively, baseline TD patients may have a higher burden of clonal hematopoiesis contributing to earlier cardiovascular disease. That ferritin was not associated with CDFS may be related to the threshold used, as ferritin >3,000 µg/L is associated with an increased risk of cardiac disease or death in another report of primarily lower risk TD patients.[23] Overall, our CDFS findings are hypothesis-generating, and should be interpreted with caution given the limited sample size in this analysis.

Preclinical data suggest that IO may increase the risk of infections, possibly through functional impairment of neutrophils, macrophages and natural killer cells.[24-27] Clinical studies in MDS support this; higher rates of infection are reported among TD *versus* TI patients,[28] and ICT may prolong the time to first infection among transfused lower

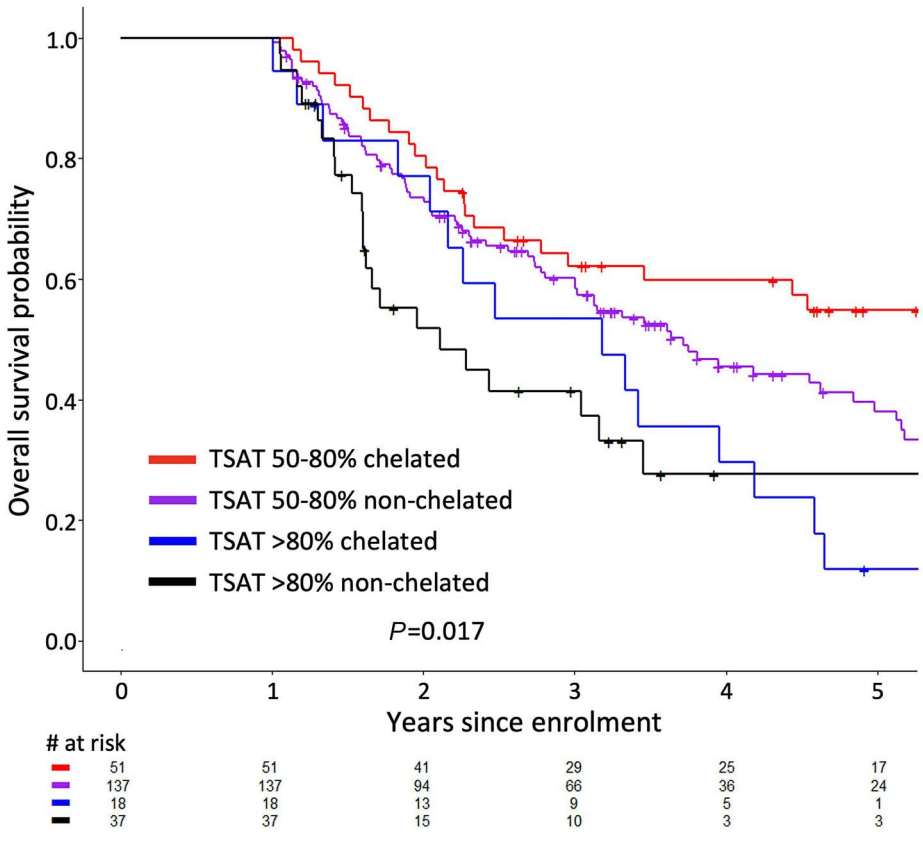

Figure 5. The effect of iron chelation therapy (ever *vs.* never). Iron chelating therapy has an attenuating effect on the adverse prognostic impact of a higher transferrin saturation (TSAT) in both intermediate (TSAT 50-80%) and high (TSAT >80%) groups, although sample sizes were limited in the tails of the Kaplan Meier survival curves.

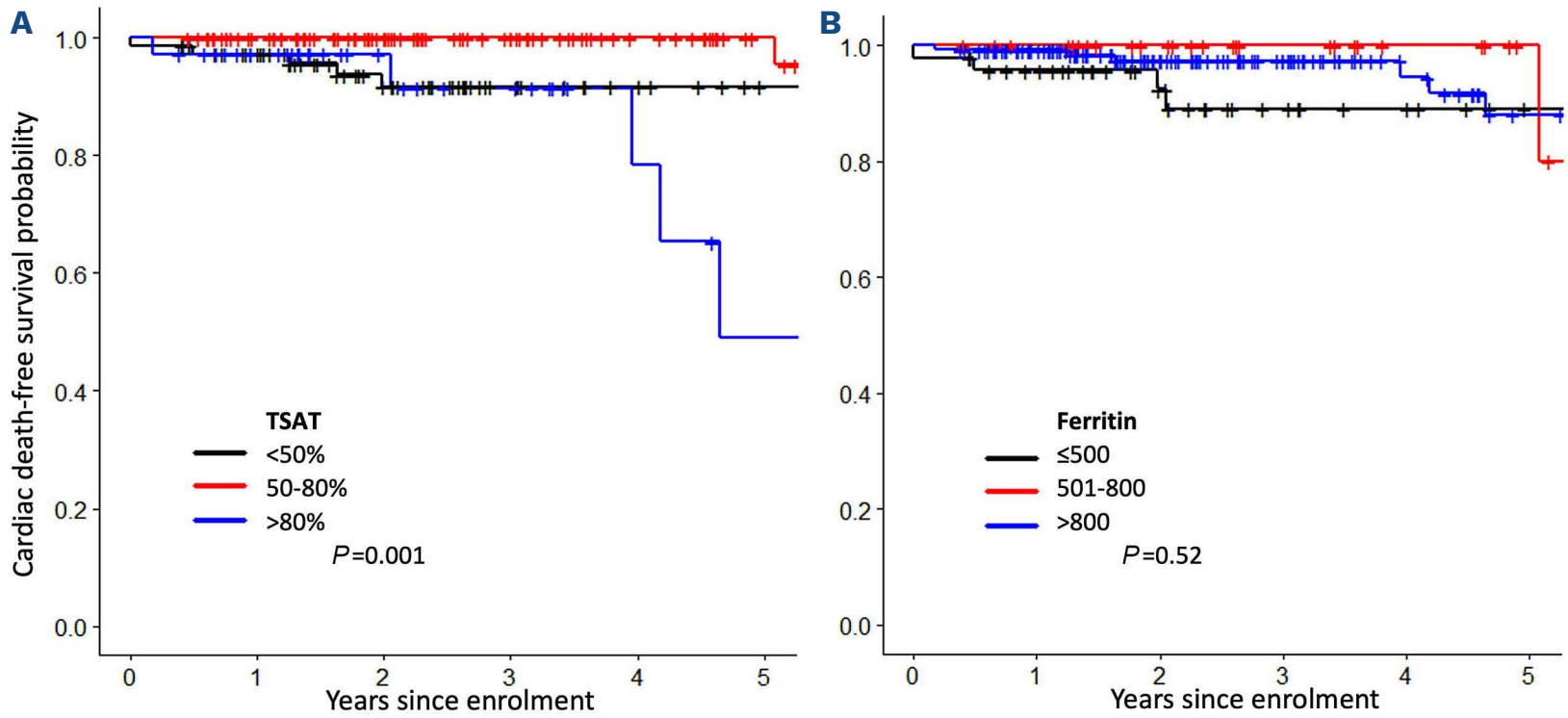

Figure 6. Cardiac death-free survival. Among patients who were transfusion-dependent at enrollment, higher mean transferrin saturation (TSAT) was associated with inferior cardiac death-free survival (A), but higher mean ferritin was not (B). Total numbers of patients at time zero in the black, red and blue groups were 75, 107 and 36 for the analysis by TSAT, and 48, 31 and 139 for the ferritin analysis, respectively.

risk patients.[29] Elevated ferritin and baseline LPI are risk factors for infection following hematopoietic stem cell transplantation for hematologic disorders.[30-35] Furthermore, NTBI and LPI promote bacterial and fungal growth in the sera of such patients.[36,37] In the latter study, elevated LPI and fungal growth *in vitro* were associated with a TSAT >75%.[37] We demonstrated an association between elevated ferritin and infectious death in MDS. Higher TSAT, however, did not show an association, suggesting that, at least in the most severe infections leading to death, ferritin may represent an acute phase reactant. Unfortunately, our registry only audits infectious deaths, not total infectious episodes, and thus only captures a small subset of infectious complications. Furthermore, cause of death was missing in 18% of patients. These factors, together with the limited number of patients with TSAT >80% (above which LPI is increased) may partially explain why TSAT did not associate with infectious death. Limitations of this study include the use of mean laboratory values in survival analyses. While a mean value over time is cumbersome to track clinically, we preferred this approach over an arbitrary static measure (i.e., at enrolment or at year 1), and were reassured by the findings that TSAT remained stable over time in TI patients and increased slightly in TD patients. Further limitations include the measurement of cardiac and infectious deaths rather than cardiac and infectious events; the latter were not reliably captured in the registry. Further study of the impact of TSAT and ferritin on cardiac and infectious morbidity is warranted. Similarly, larger studies comparing the clonal evolution and outcomes of patients with TSAT >80% who do and do not receive ICT may clarify the impact of suppressing labile plasma iron.

While a minority of patients in our cohort received ICT, TSAT should be interpreted with caution in chelated patients. The presence of free chelate in plasma may artificially increase the measured total iron binding capacity (TIBC) and thus reduce the calculated TSAT. Conversely, chelate-iron complexes may be measured as serum iron, thereby increasing the TSAT.[38] True measurement of TSAT during ICT requires alternative laboratory techniques (i.e., urea gel method) or a chelator washout. Furthermore, in a large prospective study of transfusion-dependent anemias, TSAT was not significantly reduced after one year of deferasirox.[39] Survival analyses demonstrating an attenuating effect of ICT on the impact of TSAT are limited by sample sizes.

Conclusion

In a large prospective study of MDS patients of all risk categories, TSAT >80% and ferritin >800 µg/L were clinically significant levels associated with inferior OS, PFS and LFS. The effects of transfusional IO were not restricted to lower risk patients, and a higher TSAT appears to be a better biomarker for IO toxicity than ferritin among higher risk patients. Compared to ferritin, TSAT may better reflect of the degree of oxidative stress present. Further study of the relationships between TSAT, reactive oxygen species, PFS, LFS and CDFS in MDS is warranted.

Disclosures

RB has served on BMS/Celgene advisory boards and has received research funding from TaiHo, BMS, Otsuka and Takeda. HL has served on advisory boards and received

honoraria from BMS/Celgene, Taiho and Takeda, and has received research funding support from BMS/Celgene. KY has served on advisory committees for BMS/Celgene, Takeda, Astellas, Novartis, and Pfizer, has received research funding support from Astex, Hoffman La Roche, MedImmune, Merck, Millenium and Roche/Genetech, and has received honoraria from Novartis and Pfizer. MG has served on an advisory board and received research funding from BMS/Celgene. NZ has served on advisory boards for TaiHo and BMS/Celgene. AS has served on an advisory board for BMS/Celgene. MS has received research funding support from BMS/Celgene, TaiHo, and Takeda, and has served on a BMS/Celgene advisory board. BL has served on advisory boards and received honoraria from BMS/Celgene, Taiho, Celgene and Otsuka. EH has served on advisory boards and received honoraria from BMS/Celgene. NF has served on advisory boards and received honoraria from BMS/Celgene and has served on an advisory board for Takeda. TN has served on advisory boards and received honoraria from BMS/Celgene, Taiho, and Otsuka. MK has served on a BMS/Celgene advisory board. JS has served on an advisory board and received honoraria from BMS/Celgene. ME has served on the board of directors and advisory committees for BMS/Celgene, and has received research funding support from BMS. RD has received research funding support from and has served on an advisory board for BMS/Celgene. All remaining authors have no conflicts of interest to disclose.

Contributions

RB overseas the Canadian National MDS registry. MG, NZ, MK, MiS, GC, BL, DK, ES, NF, AS, KY, JS, TN, RD, ME, VB, BH, LM, LC, HL and RB contributed patient data to the registry. MoS and AP provided administrative support for the MDS registry. JT, RB and HL designed the study. LZ performed data analysis. JT wrote the manuscript. RB, HL and LZ edited the manuscript. RB and HL co-supervised the study.

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Data-sharing statement

Requests for original data can we sent to the corresponding author

References

1. Malcovati L, Porta MG, Pascutto C, et al. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol. 2005;23(30):7594-603.
2. Malcovati L, Porta MGD, Cazzola M. Predicting survival and leukemic evolution in patients with myelodysplastic syndrome. Haematologica. 2006;91(12):1588-1590.
3. Hiwase DK, Singhal D, Strupp C, et al. Dynamic assessment of RBC-transfusion dependency improves the prognostic value of the revised-IPSS in MDS patients. Am J Hematol. 2017;92(6):508-514.
4. de Swart L, Crouch S, Hoeks M, et al. Impact of red blood cell transfusion dose density on progression-free survival in patients with lower-risk myelodysplastic syndromes. Haematologica. 2020;105(3):632-639.
5. de Swart L, Reiniers C, Bagguley T, et al. Labile plasma iron levels predict survival in patients with lower-risk myelodysplastic syndromes. Haematologica. 2018;103(1):69-79.
6. de Souza GF, Barbosa MC, Santos TE, et al. Increased parameters of oxidative stress and its relation to transfusion iron overload in patients with myelodysplastic syndromes. J Clin Pathol. 2013;66(11):996-998.
7. Okabe H, Suzuki T, Uehara E, Ueda M, Nagai T, Ozawa K. The bone marrow hematopoietic microenvironment is impaired in iron-overloaded mice. Eur J Haematol. 2014;93(2):118-128.
8. de Swart L, Hendriks JC, van der Vorm LN, et al. Second international round robin for the quantification of serum non-transferrin-bound iron and labile plasma iron in patients with iron-overload disorders. Haematologica. 2016;101(1):38-45.
9. Buckstein R, Wells RA, Zhu N, et al. Patient-related factors independently impact overall survival in patients with myelodysplastic syndromes: an MDS-CAN prospective study. Br J Haematol. 2016;174(1):88-101.
10. Malcovati L, Germing U, Kuendgen A, et al. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol. 2007;25(23):3503-3510.
11. Platzbecker U, Fenaux P, Adès L, et al. Proposals for revised IWG 2018 hematological response criteria in patients with MDS included in clinical trials. Blood. 2019;133(10):1020-1030.
12. Leitch HA, Parmar A, Wells RA, et al. Overall survival in lower IPSS risk MDS by receipt of iron chelation therapy, adjusting for patient-related factors and measuring from time of first red blood cell transfusion dependence: an MDS-CAN analysis. Br J Haematol. 2017;179(1):83-97.
13. Pereira A, Nomdedeu M, Aguilar JL, et al. Transfusion intensity, not the cumulative red blood cell transfusion burden, determines the prognosis of patients with myelodysplastic syndrome on chronic transfusion support. Am J Hematol. 2011;86(3):245-250.
14. Messa E, Gioia D, Bertassello C, et al. Prognostic value of serum ferritin, transferrin saturation and C- reactive protein at diagnosis in myelodysplastic syndrome patients: analysis of patients from the MDS Piedmont Registry. Blood. 2011;118(21):3807-3807.
15. Kim CH, Leitch HA. Iron overload-induced oxidative stress in myelodysplastic syndromes and its cellular sequelae. Crit Rev Oncol Hematol. 2021;163:103367.

16. Kikuchi S, Kobune M, Iyama S, et al. Improvement of iron-mediated oxidative DNA damage in patients with transfusion-dependent myelodysplastic syndrome by treatment with deferasirox. Free Radic Biol Med. 2012;53(4):643-648.
17. Adrianzen Herrera D, Pradhan K, Snyder R, et al. Myelodysplastic syndromes and the risk of cardiovascular disease in older adults: a SEER-medicare analysis. Leukemia. 2020;34(6):1689-1693.
18. Brunner AM, Blonquist TM, Hobbs GS, et al. Risk and timing of cardiovascular death among patients with myelodysplastic syndromes. Blood Adv. 2017;1(23):2032-2040.
19. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med 2017;377(2):111-121.
20. Wong CAC, Leitch HA. Delayed time from RBC transfusion dependence to first cardiac event in lower IPSS risk MDS patients receiving iron chelation therapy. Leuk Res. 2019;83:106170.
21. Lyons RM, Marek BJ, Paley C, et al. Comparison of 24-month outcomes in chelated and non-chelated lower-risk patients with myelodysplastic syndromes in a prospective registry. Leuk Res. 2014;38(2):149-154.
22. Angelucci E, Li J, Greenberg P, et al. Iron chelation in transfusion-dependent patients with low- to intermediate-1-risk myelodysplastic syndromes: a randomized trial. Ann Intern Med. 2020;172(8):513-522.
23. Malcovati L, Della Porta MG, Strupp C, et al. Impact of the degree of anemia on the outcome of patients with myelodysplastic syndrome and its integration into the WHO classification-based Prognostic Scoring System (WPSS). Haematologica. 2011;96(10):1433-1440.
24. Nairz M, Haschka D, Demetz E, Weiss G. Iron at the interface of immunity and infection. Front Pharmacol. 2014;5:152.
25. Cantinieaux B, Janssens A, Boelaert JR, et al. Ferritin-associated iron induces neutrophil dysfunction in hemosiderosis. J Lab Clin Med. 1999;133(4):353-361.
26. Barton Pai A, Pai MP, Depczynski J, McQuade CR, Mercier RC. Non-transferrin-bound iron is associated with enhanced staphylococcus aureus growth in hemodialysis patients receiving intravenous iron sucrose. Am J Nephrol. 2006;26(3):304-309.
27. Gomes-Pereira S, Rodrigues PN, Appelberg R, Gomes MS. Increased susceptibility to Mycobacterium avium in hemochromatosis protein HFE-deficient mice. Infect Immun. 2008;76(10):4713-4719.
28. Goldberg SL, Chen E, Corral M, et al. Incidence and clinical complications of myelodysplastic syndromes among United States Medicare beneficiaries. J Clin Oncol. 2010;28(17):2847-2852.
29. Wong CAC, Wong SAY, Leitch HA. Iron overload in lower international prognostic scoring system risk patients with myelodysplastic syndrome receiving red blood cell transfusions: Relation to infections and possible benefit of iron chelation therapy. Leuk Res. 2018;67:75-81.
30. Kataoka K, Nannya Y, Hangaishi A, et al. Influence of pretransplantation serum ferritin on nonrelapse mortality after myeloablative and nonmyeloablative allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2009;15(2):195-204.
31. Sivgin S, Baldane S, Kaynar L, et al. Pretransplant serum ferritin level may be a predictive marker for outcomes in patients having undergone allogeneic hematopoietic stem cell transplantation. Neoplasma. 2012;59(2):183-190.
32. Tachibana T, Tanaka M, Takasaki H, et al. Pretransplant serum ferritin is associated with bloodstream infections within 100 days of allogeneic stem cell transplantation for myeloid malignancies. Int J Hematol. 2011;93(3):368-374.
33. Ozyilmaz E, Aydogdu M, Sucak G, et al. Risk factors for fungal pulmonary infections in hematopoietic stem cell transplantation recipients: the role of iron overload. Bone Marrow Transplant. 2010;45(10):1528-1533.
34. Pullarkat V, Blanchard S, Tegtmeier B, et al. Iron overload adversely affects outcome of allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2008;42(12):799-805.
35. Wermke M, Eckoldt J, Götze KS, et al. Enhanced labile plasma iron and outcome in acute myeloid leukaemia and myelodysplastic syndrome after allogeneic haemopoietic cell transplantation (ALLIVE): a prospective, multicentre, observational trial. Lancet Haematol. 2018;5(5):e201-e210.
36. von Bonsdorff L, Sahlstedt L, Ebeling F, Ruutu T, Parkkinen J. Apotransferrin administration prevents growth of Staphylococcus epidermidis in serum of stem cell transplant patients by binding of free iron. FEMS Immunol Med Microbiol. 2003;37(1):45-51.
37. Petzer V, Wermke M, Tymoszuk P, et al. Enhanced labile plasma iron in hematopoietic stem cell transplanted patients promotes Aspergillus outgrowth. Blood Adv. 2019;3(11):1695-1700.
38. Ikuta K, Ito S, Tanaka H, et al. Interference of deferasirox with assays for serum iron and serum unsaturated iron binding capacity during iron chelating therapy. Clin Chim Acta. 2011;412(23-24):2261-2266.
39. Porter JB, El-Alfy M, Viprakasit V, et al. Utility of labile plasma iron and transferrin saturation in addition to serum ferritin as iron overload markers in different underlying anemias before and after deferasirox treatment. Eur J Haematol. 2016;96(1):19-26.