Iron overload in haematopoietic stem cell transplantation children and relation to organ damage and survival

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SUMMARY

Adult patients with high serum ferritin have an increased risk of organ toxicity and iron chelation before stem cell transplant might be an option. We report our experience in 58 paediatric patients (excluding patients with haemoglobinopathy and hyper-transfused patients) undergoing allogeneic stem cell transplant between 2007 and 2012. Serum ferritin pre-transplant was highly variable (mean: 932 μg/L) and related to a number of PRC transfusions. Eighteen of 58 patients had ferritin level >1000μg/L before transplant. Eight patients suffered from transplant-related mortality. We found no correlation between transplant-related mortality and pre-transplant serum ferritin (p=0.67). Seven patients developed veno-occlusive disease, reversible in all cases. We did not find a correlation between serum ferritin and veno-occlusive disease, graft-versus-host disease or relapse. The evolution of ferritin post-transplant shows a spontaneous lowering of ferritin in the first two years after haematopoietic stem cell transplantation to normal range. An association between serum ferritin and elevated AST/ALT at 12 and 24 months was noted and follow-up concerning possible liver damage in patients with a persistently high serum ferritin is recommended. This study concludes that high serum ferritin has no influence on transplant-related mortality in children, chronically poly-transfused patients excluded. Although chelation is already used in paediatric HSCT, there is insufficient, current evidence to do so.
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INTRODUCTION

Haematopoietic stem cell transplantation (HSCT) is currently the only treatment with curative potential for various benign and malignant disorders. Because transplant-related mortality (TRM) is the primary adverse effect of stem cell transplantation (SCT), several prognostic factors have been described to predict transplant outcome. In our centre, TRM has declined from 32% in between 1989-1999 to 15% in the last ten years.[1] Better therapy for infectious complications and better knowledge of non-infectious complications might be a cause. Veno-occlusive disease (VOD) is a well-known complication in the early post-SCT period and a life-threatening disease. Factors possibly influencing the risk of VOD include the use of busulfan in the conditioning regimen, the underlying disease such as metabolopathy and viral or non-viral hepatic disease. Hepatic haemosiderosis due to iron overload is an important problem in candidates and survivors of SCT and has been reported to have an adverse effect on long term survival in adults.[2,5] Iron is an essential element in the organism and has a crucial role in several biochemical reactions including oxygen transport and electron transfer. However, excess of iron can cause tissue damage through the production of free radicals.[3,4] The primary toxic form of iron, non-transferrin-bound iron (NTBI), is detected

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Keywords: children, ferritin, iron overload, SCT, stem cell transplantation, TRM.

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TABLE 1. Patient characteristics.

	Serum ferritin pre-transplant		Total population	P
	≤1000μg/L	>1000μg/L		
Sex F M	N (%) 14 (35) 26 (65)	N (%) 8 (44.4) 10 (55.6)	(N%) 22 (37.9) 36 (62.1)	0.56
Age at transplant N Min-max (median)	 40 0-17 (6)	 18 0-17 (6)	 58 0-17 (6)	0.44
Diagnosis ALL AML High-grade MDS Other	N (%) 5 (12.5) 2 (5) 4 (10) 29 (72.5)	N (%) 6 (33.3) 7 (38.9) 1 (5.6) 4 (22.2)	N (%) 11 (19) 9 (15.5) 5 (8.6) 33 (56.9)	0.0002
Time between diagnosis and HSCT (months) N Min-max (median)	 40 0-209 (5)	 18 1-73 (8)	 58 0-209 (6)	0.34
Relapse Y N	N (%) 9 (22.5) 31 (77.5)	N (%) 7 (38.9) 11 (61.1)	N (%) 16 (27.6) 42 (72.4)	0.22
Mortality Y N	N (%) 12 (30) 28 (70)	N (%) 10 (55.6) 8 (44.4)	N (%) 22 (37.9) 36 (62.1)	0.083
TRM Y N	N (%) 5 (12.5) 35 (87.5)	N (%) 3 (16.7) 15 (83.3)	N (%) 8 (13.8) 50 (86.2)	0.69
Acute GvHD Y N	N (%) 11 (27.5) 29 (72.5)	N (%) 5 (27.8) 13 (72.2)	N (%) 16 (27.6) 42 (72.4)	1
Chronic GvHD Y N	N (%) 2 (5) 38 (95)	N (%) 1 (5.6) 17 (94.4)	N (%) 3 (5.2) 55 (94.8)	1
VOD Y N	N (%) 4 (10) 36 (90)	N (%) 3 (16.7) 15 (83.3)	N (%) 7 (12.1) 51 (87.9)	0.17
Number of transfusions pre-transplant N Min-max (median)	 40 0-54 (2)	 18 1-61 (15)	 58 0-61 (4)	<0.0001
Number of transfusions post-transplant N Min-max (median)	 40 0-38 (9)	 18 1-105 (9)	 58 0-105 (9)	0.76
Liver biopsy J N	N (%) 1 (2.5) 39 (97.5)	N (%) 4 (22.2) 14 (77.8)	N (%) 5 (8.6) 53 (91.4)	0.03
Liver NMR T2* J N	N (%) 1 (2,5) 39 (97,5)	N (%) 4 (22,2) 14 (77,8)	N (%) 5 (8,6) 53 (91,4)	0.03
Cardial NMR T2* J N	N (%) 1 (2.5) 39 (97.5)	N (%) 1 (5.6) 17 (94.4)	N (%) 2 (3.5) 56 (96.6)	0.53

HSCT: haematopoietic stem cell transplantation, TRM: transplant related mortality, GvHD: graft-versus-host disease, VOD: veno-occlusive disease.

when transferrin saturation exceeds 70% to 80%.[2] In transfusional overload, iron is primarily located in the reticular endothelial system (RES) and later accumulates in the parenchyma, while possibly causing organ damage.[6,8] Iron overload can be detected in serum and with the use of imaging techniques. Because ferritin is an acute-phase protein, it does not always reliably predict total body iron stores, whereas NTBI might be a more specific indicator of iron overload.[3,4] Nonetheless, serum ferritin (SF) remains the most cost-effective and widely available means of estimating iron overload defined as SF concentration of more than 1000 μg/L.[3]
The most common complication of iron overload is liver damage and the risk of VOD, although others find no correlation with elevated SF.[2,7,11,14] Other complications possibly related to iron overload are the risk of heart disease and death.[4,7]
The objective of the present study was to determine the effect of iron overload, as measured using SF concentration, on TRM and survival, in 58 children undergoing HSCT. The primary objective was to examine the possible influence of SF on transplant outcome and on TRM. We also examined the spontaneous evolution of SF up to 24 months post-transplant.

SUBJECTS AND METHODS

Patients: Sixty-five allogeneic HSCT paediatric patients, treated at the Ghent University Hospital (Belgium) during the period 2007-2012 were retrospectively studied. Four patients had a second transplant. Poly-transfused patients (thalassemia major: one and aplastic anaemia: two) who had chelation therapy before their SCT were excluded. Fifty-eight patients were studied covering 62 transplants. Study approval from the ethical committee and the informed consent of the patients and/or legal guardians were obtained for all patients.
Methods: SF was used to measure iron overload. Because SF is influenced by other factors (e.g., infection), free iron (FE), C reactive Protein (CRP), transferrin and total iron-binding capacity (TIBC) were also evaluated. Liver function was monitored using AST, ALT, γGT and AF.
Analysis: Patients were followed until December 2014, at least 24 months of follow-up after the last patient inclusion. To compare the current literature most adequately with our results, we dichotomised our patients using SF=1000 μg/L as the cut-off value. For descriptive statistics, the two groups were compared using Fisher's Exact Test for categorical variables and the Mann-Whitney U test for (semi-)continuous variables. Initially, we performed a logistic regression with TRM as outcome variable and three separate models for the predictors (SF pre-transplant (>1000 μg/L vs ≤ 1000 μg/L), CRP and graft-versus-host disease [GvHD]). Additionally, a multiple logistic regression with SF pre-transplant and CRP as predictors was applied.
Fisher's Exact Test was used to detect a possible association between AST (cut-off: 50 U/L) and ALT (cut-off: 40 U/L) and SF higher or lower than 1000 μg/L. To study the evolution of SF the SF concentrations were log-transformed after which the distribution was approximately normal. Since data were longitudinal (and thus correlated), mixed linear regression was applied with log (SF concentrations) as outcome variable and time as predictor variable.
A Kaplan-Meier curve was created to evaluate overall survival (OS) in the entire population. Mann-Whitney U tests were used to compare the number of transfusions in our dichotomised population.

RESULTS

PATIENT CHARACTERISTICS

Files of 58 paediatric patients who underwent an allo-HSCT were studied (*Table 1*). Thirty-six were male and the average age at transplant was seven years (median: 6, range: 0-17). The main indications for HSCT were ALL (11/58), AML (9/58) and high-grade MDS (5/58). Thirty-three transplants were performed for various other malignant and non-malignant diseases. Stem cells from bone marrow (BM) were the preferred cell source (65%) and most donors were matched unrelated donors (MUD 33/58). Four patients received stem cells from a haploidentical donor, mostly SCID patients.
Patients mainly underwent myeloablative conditioning (MAC; 50/58). Reduced intensity conditioning (RIC) was used in three second transplants. Five SCID patients received no conditioning. Total body irradiation (TBI) was given to twelve patients as part of their conditioning regimen.
Follow-up lasted two to eight years. During this period, 22 of 58 patients died (mortality: 37.9%). TRM (death not due to relapse of disease) was responsible for 8 of 22 deaths (36.4%). TRM was caused by GvHD in two patients, viral infections in three (once encephalitis e causa ignota, once adenovirus, once HHV6 encephalitis), bacterial sepsis in two and mucormycosis in one. TRM occurred at an average of four months post-transplant (range: one to thirteen months).
A relapse of the disease was observed in sixteen patients (relapse rate: 27.6%), out of which fourteen died.
Acute GvHD was seen in sixteen patients of whom four had a grade 4 GvHD. Three patients suffered from chronic GvHD.
VOD occurred in seven patients (12%) of which two were severe. VOD was reversible with supportive therapy in all patients.
The OS of the study population is shown in the Kaplan-Meier Curve (*Figure 1*).

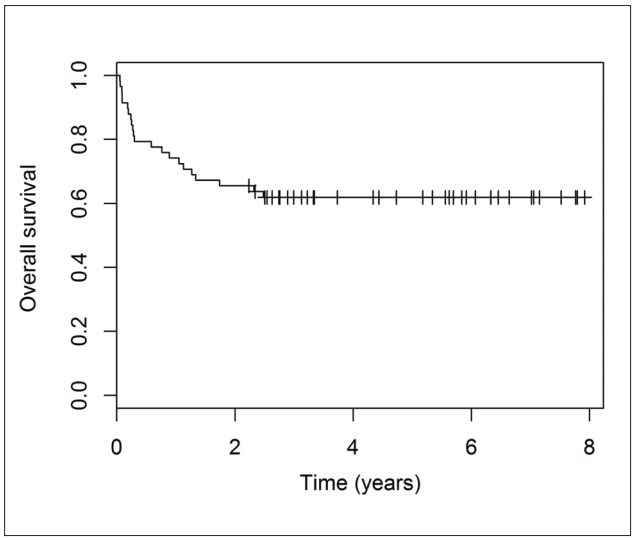

FIGURE 1. Kaplan-Meier Curve: overall survival (OS) 2007-2014.

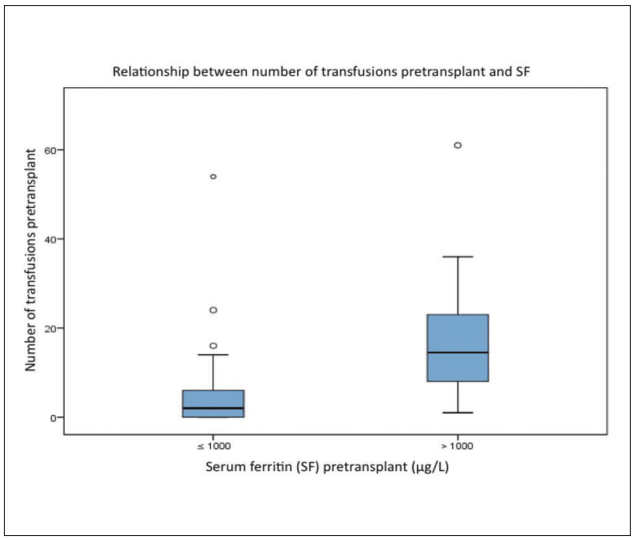

FIGURE 2. The relation between the number of transfusions pre-stem cell transplantation and serum ferritin (p<0.0001).

IRON OVERLOAD AND TRANSPLANT RELATED MORTALITY

In the patient population, the level of SF was highly variable with a mean of 932 µg/L (range: 15-11695) pre-transplant and related to the number of PRC transfusions (Mann-Whitney U, p<0.001) (*Figure 2*). In eighteen patients, SF pre-SCT was higher than 1000 µg/L. The highest SF concentrations were observed at three months post-SCT (geometric mean: 475 µg/L, 95%-CI: 281-803). Our study of the evolution of SF post-transplant shows a spontaneous decline of ferritin in the first two years after HSCT to a geometric mean of SF of 215 µg/L (95%-CI: 127-365) at twelve months and 168 µg/L (95%-CI: 97-292) at 24 months post-SCT (*Figure 3*).

TRM was present in five of forty (12.5%) patients with pre-transplant SF ≤1000 µg/L and in three of eighteen (16.7%) patients with SF >1000 µg/L. We found no significant association between TRM and SF (p= 0.67; (*Figure 4*). The estimated odds ratio (OR) for TRM was 1.4 for patients with SF level >1000 µg/L compared with patients with SF levels ≤1000 µg/L (95%-CI: 0.3-6.62).

SF pre-transplant also had no significant effect on TRM when corrected for pre-transplant CRP (p=0.39). This correction was performed to exclude infection as a cause of elevated SF. The estimated OR for TRM are 2.1 times higher in patients with SF >1000 µg/L compared with those with SF ≤1000 µg/L (OR=2.1; 95%-CI: 0.4-11.3).

IRON OVERLOAD AND ORGAN DAMAGE

Liver damage as the most prevalent complication of iron overload was evaluated by means of elevated ALT (>40 U/L) or AST (>50 U/L) in serum of SCT patients. There was no association between ALT/AST and SF in the first year post-SCT. However, a significant association between SF and liver transaminases at twelve months post-SCT (Fisher's Exact Test, p=0.03) and 24 months post-SCT (p=0.005) was noted.

Seven patients developed VOD, which was reversible with supportive therapy. This study did not find an association between SF and VOD (p=0.17), GvHD (p=1) or disease relapse (p=0.22).

Seven patients underwent imaging for liver or heart using T2*MRI or had a liver biopsy. Almost all of them had a persistent high SF. In five patients mild to serious iron overload in the liver was confirmed. T2*MRI of the heart (two patients) showed no signs of cardiac overload.

DISCUSSION

The objective of the present study was to assess the predictive role of iron status, defined as SF, in TRM and mortality in children who underwent a haematopoietic allogeneic SCT. The retrospective data showed no relation between high SF and TRM in the paediatric population. OS was 62% at five years and was independent of SF. This result in the paediatric population is in contrast with the reports in adults where a strong correlation between pre-transplant SF and survival was described.[10,11] Sucak reported in 250 adults who underwent autologous and allogeneic SCT that high SF (>500 µg/L) was related with significant more morbidity including post-SCT infections and SOS (VOD).[15] TRM accounts for 10-30% of deaths in allogeneic HSCT, depending on pathology, remission status, age and complications.[1] In the study population, TRM was observed in 14%. One of the most feared

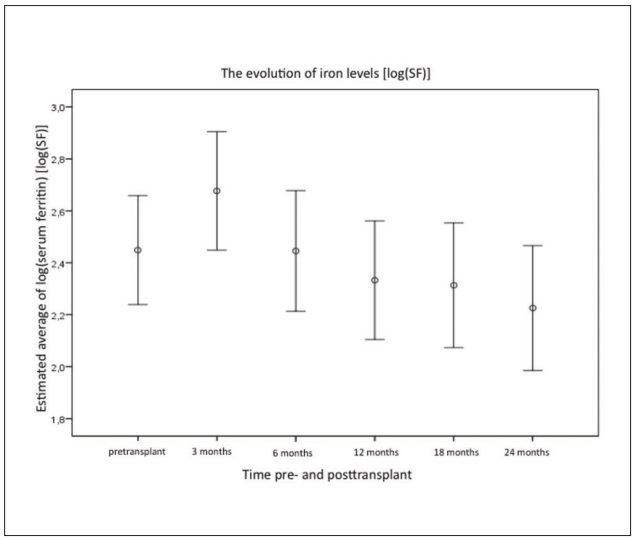

FIGURE 3. The natural evolution of serum ferritin: average of log (serum ferritin; [log(SF)]) in function of time pre- and post-transplant.

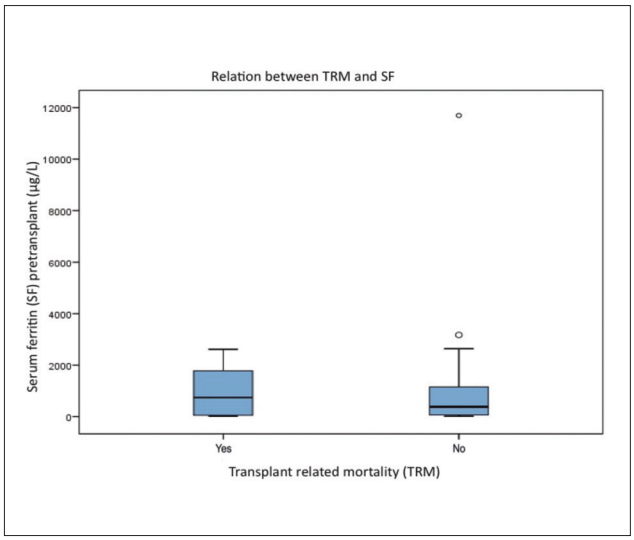

FIGURE 4. The relation between transplant related mortality (TRM) and serum ferritin (p:0.67).

complications during transplant is the occurrence of hepatic VOD, and pre-transplant liver tissue damage (due to iron overload e.o.) is a known risk factor for VOD. In this study's cohort, seven patients developed VOD during transplant but none died of VOD and all were cured with supportive therapy. One patient died of mucormycosis following transplant; the SF pre-transplant was <1000/µL. The role of transfusional iron overload as a possible co-risk factor for the development of mucormycosis post-SCT has been described.[16]

The reason of this discrepancy between the results in children and adults is not clear. Liver function might be better and damage can be reversible in liver tissue in small children compared with adults. The amount of iron overload in adults might be much higher, not only because of the more frequent PRC transfusions, but also because of the underlying disease.

This study also wanted to examine the spontaneous evolution of rSF during the post-SCT period. SF reached a peak at three months post-SCT and declined gradually after that with a mean of 1004 µg/L at twelve months and had a further spontaneous decline to 445 µg/L at 24 months. The results confirm that elevated SF levels decrease spontaneously over the first two years post-SCT. This finding is important as it may lessen the need for chelation, a therapy that still has to undergo a cost-effectiveness analysis.[17]

Another point of interest was the link between permanently elevated SF and a rise in transaminases (ALT, AST) at one year post-SCT and beyond. SF remains the most commonly used and cost-effective way to determine iron overload, but it also has its limitations. In the future, T2*MRI and new parameters, like soluble transferrin receptor (sTFR), can provide a more clear view of iron overload as was recently described.[17,18] We suggest determining SF levels as well as AST and ALT on a regular base in the first year post-transplant. In patients in which both parameters are elevated at twelve months post-SCT, a liver biopsy and/or liver T2*MRI should be conducted.

Information on iron overload (pathophysiology, evolution, effect on organs, chelation) in children is not only limited, but often results from research conducted in patients with chronic anaemia (e.g., haemoglobinopathy) who have undergone transfusions for many years. Hepatic and/or cardiac damage in poly-transfused children occurs after more than ten years.[17] Long-lasting iron overload and damage in thalassemia and sickle cell anaemia patients is clearly different from only temporary, limited and reversible overload in patients who receive transfusions for a limited time period before an allogeneic HSCT. Therefore, we cannot simply transfer results from one group to the other, and additional research in the latter group is urgently needed.

The retrospective data showed no relation between high SF and TRM in the paediatric population. We will acknowledge the limitations of this study as a retrospective, single-centre study in a small group of 58 paediatric patients with heterogeneous pathologies. This heterogeneity makes it harder to apply the results to every paediatric patient.

CONCLUSION

We conclude that high SF (>1000 µg/L) has no influence on TRM in children, chronically poly-transfused children

KEY MESSAGES FOR CLINICAL PRACTICE

1. **High serum ferritin pre-transplant has no correlation with transplant related mortality in children, chronically poly-transfused children excluded.**
2. **This study shows a spontaneous decline of the serum ferritin level in the first two years after a successful transplantation to a normal range. However, a persistently high serum ferritin at 24 months post-SCT warrants further investigation for possible liver damage.**
3. **The value of chelation therapy based on high serum ferritin levels pre- and early post-transplant in children is not yet clear.**
4. **Guidelines for detection of iron overload and indications for chelation therapy in children undergoing stem cell transplant are needed.**

excluded. We did, however, mark a correlation between high transaminases and SF in the later period post-transplant. Therefore, we strive for more follow-up concerning possible liver damage in patients who show a persistently high SF. This should be done using T2*MRI and/or liver biopsy. Although chelation is already used in paediatric HSCT, there is insufficient current evidence to do so. Clear guidelines for detection and possible treatment of iron overload in children undergoing allogeneic HSCT are needed.

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