Pre-transplantation serum ferritin as a prognostic marker in allogeneic haemopoietic stem cell transplant patients in a tertiary care hospital in Malaysia

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ABSTRACT

Background. The principal cause of iron overload in patients with haematological malignancies is recurrent red cell transfusions for anaemia. The serum ferritin level reflects the iron burden in the body, in the absence of inflammation or liver disease. In Malaysia, data are lacking on the association between pre-transplant serum ferritin levels and outcome after allogeneic haemopoietic stem cell transplant.

Methods. We did a cross-sectional study using retrospective data of 106 post-allogeneic haemopoietic stem cell transplant patients (HLA-matched sibling) with haematological malignancies at Hospital Ampang to determine the relationship between pre-transplant serum ferritin levels and post-transplant outcome, post-transplant complications and survival time. Patients were divided into two groups according to the iron status: serum ferritin level ≥1000 μg/L (iron overload) and <1000 μg/L.

Results. The median age for patients was 30.5 (18–58) years. The median pre-transplantation serum ferritin level and the prevalence of pre-transplantation iron overload were 2423 (408.2–7664) μg/L and 87.5%, respectively. No significant association was found between iron status and demographic factors, type of haematological malignancy and post-transplant complications. Although insignificant, patients with iron overload had a shorter survival time (36 months) compared to those with no iron overload (40 months). There was also no significant association between the iron status and post-transplant outcome. Significant post-transplant complications associated with post-transplant outcome were the need for total parenteral nutrition (TPN) (p=0.014) and chronic graft-versus-host disease (GVHD) (p=0.008). Similarly, significant associations were found between age group (p=0.003), TPN (p=0.035) and chronic GVHD (p=0.012) with survival time using Kaplan–Meir analysis. However, after Cox regression, only age group was found to be significantly associated with survival time (p=0.014).

Conclusion. Serum ferritin is an acute phase reactant and its levels increase in the presence of tissue necrosis and inflammation. Both these events occur in haematological malignancies. Although serum ferritin level is a non-invasive, relatively cost-effective, widely available and practical indicator of iron status, it is not specific to iron overload. Therefore, a true association between the serum ferritin level and iron burden is problematic in patients with haematological malignancies.

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INTRODUCTION

The principal cause of iron overload in patients with haematological malignancies is recurrent red cell transfusions for anaemia.[1] The estimation of iron overload is currently based on the level of serum ferritin, but in allogeneic haemopoietic stem cell transplant (alloHSCT) recipients, many confounding factors such as inflammation, ineffective erythropoiesis and liver disease can lead to an overestimation of ferritin levels.[2] Before transplantation and in the early post-transplant period, these patients often receive multiple blood transfusions for anaemia. This is the main cause for hyperferritinaemia, defined as serum ferritin level ≥1000 μg/L because abnormalities of iron-related liver function test have been reported above this level.[2]

Besides transfusion of red cells, ineffective erythropoiesis and release of iron from the bone marrow and liver due to cell damage caused by toxicity of the conditioning regimen also disturb iron homeostasis.[3] These changes lead to a collection of macrophage iron, which is subsequently transported into the plasma by ferroportin. Non-transferrin bound iron (NTBI), which is responsible for the abnormal pattern of iron distribution in plasma, can be highly toxic by facilitating conversion of hydrogen peroxide into free radicals when the capacity of plasma transferrin to bind iron is exceeded.[1,3] Moreover, its redox-active, cell-penetrating component, labile plasma iron (LPI) emerges.[1] NTBI and LPI are mediators of the deleterious effects of iron overload, first giving rise to a pro-oxidant state, which then causes tissue damage and organ compromise by oxidation of proteins, peroxidation of membrane lipids and modification of nucleic acids; thus increasing the risk of post-transplant complications.[4] Since excess iron builds up in all organs, this eventually leads to multi-organ failure.[4] The risk of iron-related organ toxicity is determined by

the rate and amount of iron burden.[4] We aimed to determine the association between pre-transplantation serum ferritin levels, post-transplant outcome, post-transplant complications and survival time in post-alloHSCT patients in Hospital Ampang, a tertiary haematology centre in Malaysia.

METHODS

Study design

In this cross-sectional study, we used retrospective data of 106 alloHSCT patients (HLA-matched sibling) ≥18 years old with acute leukaemia (acute myeloid leukaemia [AML], acute lymphoid leukaemia [ALL] or myelodysplastic syndrome [MDS]) from 2008 to 2011. The study was done at the Haematology Department, Hospital Ampang to determine the relationship between pre-transplant serum ferritin levels and post-transplant outcome (alive or dead), post-transplant complications and survival time (survival of patients in months post-transplant). The sample size was calculated using the prevalence of deceased transfusion-dependent patients whose deaths were attributed to iron overload, 97% having serum ferritin levels ≥1000 µg/L.[5] The patients were divided into two groups according to their iron status: serum ferritin level ≥1000 µg/L (iron overload) and <1000 µg/L. Ten patients who did not have data on iron status were excluded from the study.

Data collection

Pre- and post-clinical and laboratory data were obtained electronically from the patients and laboratory information systems, respectively and recorded in a proforma. Confidentiality of patients' identity was ensured. Laboratory data included pre-transplant levels of serum ferritin. Other information obtained electronically included demographic factors (gender, age and ethnicity) and clinical details (type of haematological malignancy, post-transplant outcome, post-transplant complications and survival time).

Laboratory measurements

Serum ferritin levels were measured using the chemiluminescent microparticle immunoassay (CMIA) technology on Architect system (Abbott Laboratories, Abbott Park, USA). The reference range was 21.8–274.7 µg/L for men and 4.6–204.0 µg/L for women.

Statistical analysis

Statistical calculations were done using the standard statistical software package, IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY: IBM Corp). Median and range were calculated for all non-normally distributed continuous variables. Kaplan–Meier survival analysis and Cox proportional hazards regression models were used to analyse the association between all the variables and survival time. Associations between variables and post-transplant outcomes were determined by chi-square test, Fisher exact test and SAS exact contingency table. In all statistical analyses, $p<0.05$ (95% confidence interval) was considered to be statistically significant.

Ethics

We obtained approval to conduct the study from the Medical Research and Ethics Committee, Ministry of Health Malaysia and the Director of Hospital Ampang. Ethical approval was also obtained from the Ethics Committee for Research involving Human Subjects of Universiti Putra Malaysia.

RESULTS

The median age of 106 patients was 30.5 (range 18–58) years (Table I). The prevalence of pre-transplantation iron overload in these patients was 87.5%. The median pre-transplantation serum ferritin level was 2423 (range 408.2–7664) µg/L. We encountered six different types of complications (Table II). The majority of patients had mucositis, usually grade III. The other common complications in descending order were fever, acute graft-versus-host disease (GVHD), need for total parenteral nutrition (TPN), relapsed cases and chronic GVHD. There was no significant association between iron status and demographic factors, type of haematological malignancy and post-transplant complications.

The majority of patients who had undergone alloHSCT were still alive (*n*=79; 74.5%). However, there was no significant association between iron status and post-transplant outcome (p=0.73, Table III).

The median survival was 16.4 months (interquartile range 9.14–30.0 months, Fig. 1). Patients with iron overload had a non-significant shorter mean survival time (36.1 months, SE 2.21; 95% CI 31.74–40.4) compared to those with no iron overload (40.6 months; SE 4.58; 95% CI 31.65–49.62). However, there was no significant difference in survival between those with iron overload and those without (p=0.35).

Using the Cox proportional hazard regression model, the hazard ratio was 1.97 (95% CI 0.46–8.4). However, the association between iron status and survival time was not statistically significant (p=0.36).

Among the post-transplant complications, only TPN (p=0.014) and chronic GVHD (p=0.008) had a significant association with post-transplant outcome (Table IV).

For different grades of mucositis, significant associations were present between patients with grades I and II mucositis (p=0.009) and between grades II and IV mucositis (p=0.006) after Bonferroni correction. Patients without TPN had a longer survival time (39.6 months; SE 2.03; 95% CI 35.58–43.52) compared to those on TPN. Similarly, patients without chronic GVHD had a

TABLE I. Demographics and clinical details of the study population

Characteristic	*n* (%)
Age (years)	
<20	13 (12.3)
20–29	35 (33.0)
30–39	27 (25.5)
40–49	24 (22.6)
50–59	7 (6.6)
Gender	
Men	55 (51.9)
Women	51 (48.1)
Race	
Malay	48 (45.3)
Chinese	42 (39.7)
Indian	12 (11.3)
Bumiputra Sarawak	4 (3.8)
Type of malignancy	
ALL	38 (35.9)
AML	62 (58.5)
MDS	6 (5.7)
Iron status	
Non-iron overload (ferritin <1000 µg/L)	12 (11.3)
Iron-overload (ferritin ≥1000 µg/L)	84 (79.3)
Data not available	10 (9.4)

TABLE II. Post-transplant complications in the study population

Complication	*n* (%)
Mucositis	
No	2 (1.9)
Grade I	14 (13.2)
Grade II	27 (25.5)
Grade III	47 (44.3)
Grade IV	13 (12.3)
Missing	3 (2.8)
Fever	
Yes	93 (87.7)
No	10 (9.4)
Missing	3 (2.8)
Total parenteral nutrition	
Yes	22 (20.8)
No	81 (76.4)
Data missing	3 (2.8)
Disease relapse	
Yes	21 (19.8)
No	82 (77.4)
Data missing	3 (2.8)
Acute graft-versus-host disease	
Yes	25 (23.6)
No	78 (73.6)
Data missing	3 (2.8)
Chronic graft-versus-host disease	
Yes	9 (8.5)
No	94 (88.7)
Data missing	3 (2.8)

TABLE III. Outcome of patients undergoing allogeneic haemopoietic stem cell transplantation in relation to their iron status

Iron status	Outcome (%)	
	Alive	Dead
Overload	61 (72.6)	23 (27.4)
No overload	10 (83.3)	2 (16.7)

Data for 10 patients was not available; 8 of them were alive

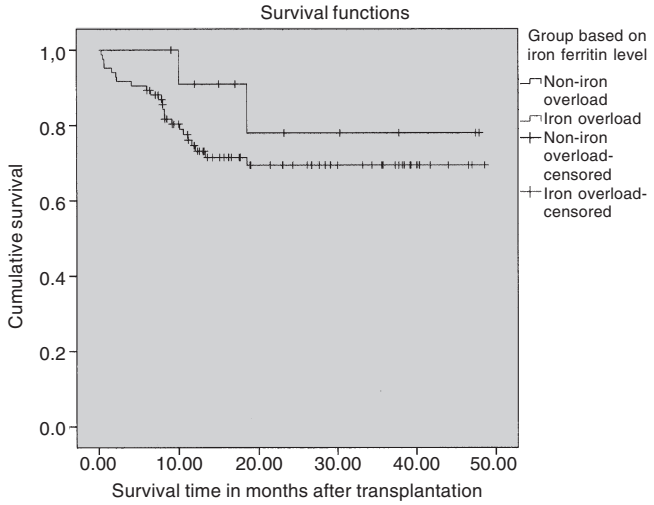

FIG 1. Survival curve of patients by iron status

TABLE IV. Distribution of post-transplant complications and outcome

Complication	Outcome (%)	
	Alive	Dead
Mucositis	75 (74.3)	26 (25.7)
Fever	68 (73.1)	25 (26.9)
Acute GVHD	18 (72.0)	7 (28.0)
Total parenteral nutrition	12 (54.5)	10 (45.5)
Relapsed disease	13 (61.9)	8 (38.1)
Chronic GVHD	3 (33.3)	6 (66.7)

GVHD graft-versus-host disease

longer survival time (38.9 months; SE 1.92; 95% CI 35.15–42.67) compared to those who developed chronic GVHD after transplantation. Using the Log Rank test, significant difference of survival time was seen between patients receiving and not receiving TPN (p=0.035) and between patients with and without chronic GVHD (p=0.012).

Further analysis was done to find significant associations between covariates (iron status, age group, TPN and chronic GVHD) and the survival time of patients after alloHSCT and their hazard ratio. Using reference category for each covariate, only age group significantly contributed to the survival time of patients. Patients who were <30 years old had the hazard of mortality decrease by more than half compared to those ≥30 years (p=0.01; HR=0.24; 95% CI 0.08–0.75). Although the iron status (p=0.61; HR=0.65; 95% CI 0.13–3.42) did not significantly affect the survival time of patients, it was clinically important that the hazard of mortality decreased when patients did not have iron overload. Patients who required TPN after alloHSCT had a 1.71-times (95% CI, 0.73–4.03) higher risk of mortality compared to those who did not require TPN. Also, patients who developed chronic GVHD after alloHSCT had a 2.40-times (95% CI 0.92–6.25) risk of mortality compared to those who did not develop chronic GVHD. However, this association between TPN and chronic GVHD with survival time, although clinically relevant, was not statistically significant (p=0.22 and p=0.07, respectively).

DISCUSSION

In our study, the prevalence of pre-transplantation iron overload in patients with haematological malignancy in Ampang Hospital, a tertiary haematology centre in Malaysia, between 2008 and 2011, was 87.5%. This observation was based on the serum ferritin level ≥1000 µg/L and was relatively high compared to previous studies in which the prevalence ranged from 32% to 58%.[1,3,6] Pullarkat *et al*. found iron overload to be an important prognostic factor for patients' survival and suggested that in a population with a high prevalence of pre-transplantation iron overload, screening for assessment of iron status should be made mandatory to prevent complications related to iron-overload, thus decreasing the risk of morbidity and mortality.[6]

According to Kataoka *et al*., patients undergoing alloHSCT for haematological malignancies in the high ferritin group had a lower overall survival than those in the low ferritin group. Multivariate analyses showed that a high pre-transplantation serum ferritin level was a significant risk factor for poor survival outcome.[7] Malcovati evaluated the prognostic value of patients with MDS developing iron overload during their follow-up.[8] In this study, a serum ferritin level of 1000 µg/L was chosen as a threshold to distinguish between mild and clinically relevant iron burden (this threshold was reached after patients received a

median of 21 units of blood transfusion). A significant association between the development of secondary iron overload and overall survival, with a hazard ratio of 1.36 for every 500 μg/L of serum ferritin increment above the threshold was noted. Moreover, it also showed that clinically significant iron overload (liver iron concentration 47 mg/g) was uncommon in patients with serum ferritin levels <1000 μg/L.[8] Following alloHSCT for acute leukaemia and MDS, raised pre-transplantation serum ferritin levels are found to be associated with poor outcome.[6] For this reason, serum ferritin has been integrated into the prognostic scoring systems for patients who have undergone alloHSCT for these two types of haematological malignancies.[9]

Our results show an association between post-transplant complications and use of TPN, chronic GVHD and between grades of mucositis. Sonis *et al*. in a multicentre study of patients undergoing HSCT showed that the extent and severity of oral mucositis was significantly associated with an increase in mortality.[10] Our study supports these findings although no significant associations were found between grades of mucositis and iron status (p=0.66) and post-transplant outcome (p=0.06), respectively. A significant difference was present when the survival time was compared between grades of mucositis. Patients with lower grades of mucositis had a longer mean survival time and vice versa. Oral mucositis may limit the patient's ability to tolerate chemotherapy or radiation therapy. It may result in dose-limiting side-effects and hence interfere with cancer treatment and outcome.[11] Compliance with treatment is affected as severe oral mucositis compromises the delivery of optimal therapy.[12] These interruptions in dosing can directly impact patient survival.[10]

We found no association between TPN and iron status although patients who received TPN had a higher incidence of iron overload (*n*=18 [90%]). There were significant associations of TPN with post-transplant outcome and survival time. TPN recipients had a higher death rate (p=0.01) and a shorter mean survival time (p=0.04) compared to non-recipients. However, we did not look for reasons for TPN exposure affecting survival outcome. While TPN is often given to patients to prevent nutritional deficit during the peri-transplant period, there is conflicting evidence to support its routine use. Few prospective randomized studies have reported increased survival and decreased relapse in patients undergoing HSCT who received TPN compared to controls,[13] while other studies showed no difference.[14]

Chronic GVHD affects up to 40%–70% of alloHSCT recipients and remains the most important cause of late transplant-related morbidity and mortality.[15,16] However, we found no association between chronic GVHD and iron status although patients with chronic GVHD had a higher incidence of iron overload (*n*=8; 88.9%). Consistent with previous observations, chronic GVHD was significantly associated with increased mortality rate (p=0.008) and shorter mean survival time (p=0.012). Although the exact mechanism of how chronic GVHD leads to death is unknown, the pathophysiology involves immunodeficiency induced by the disease itself or its immunosuppressive chemotherapy leading to death from irreversible organ dysfunction or infection.[17]

Evidence from previous studies has highlighted iron overload as a strong predictor of poor outcome after transplant. However, our study provided no support for this evidence. Although the elevated pre-transplantation serum ferritin level appeared to have an adverse impact on post-transplant outcome and survival time clinically, there was no significant association between these variables. This discrepancy of results could be due to some limitations of this study; one limitation being that there were disproportionate number of patients in the two groups—iron overload (84) versus no iron overload (12). Also, data were not available on pre-transplant serum ferritin levels for 10 patients. The second limitation was that only a one-off reading for serum ferritin level was taken for analysis, which may not exactly reveal the clinical condition. Apart from that, CRP or ESR results were not available for all patients to exclude those with inflammation-induced elevated serum ferritin levels, considering that serum ferritin is an acute-phase protein. Moreover, the variability of the finding may confound the effects of covariates on outcomes. Lastly, this being a cross-sectional epidemiological study, we did not adjust for potential unmeasured confounders; thus, a temporal relationship could not be established.

Conclusion

Serum ferritin is an acute phase reactant and its levels increase in the presence of tissue necrosis and inflammation. Both these events occur in haematological malignancies. Although the serum ferritin level is a non-invasive, relatively cost-effective, widely available and practical indicator of iron status, it is not specific to iron overload. Therefore, a true association between serum ferritin level and iron burden is problematic in patients with haematological malignancies. To overcome the limitations of this study, larger prospective multicentre studies with longer follow-up would help in ascertaining the association between serum ferritin and iron burden.

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Conflict of interest. None declared.

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