

Iron overload

Iron overload might increase transplant-related mortality in haematopoietic stem cell transplantation

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Summary:

Iron overload (IO) is associated with free radical generation and tissue damage. Our main objective was to ascertain if very high levels (VHL) of ferritin ($\geq 3000 \mu\text{g/l}$) and transferrin saturation (TS) $\geq 100\%$ during conditioning had an impact on overall survival (OS) and transplant-related mortality (TRM) after a haematopoietic stem cell transplantation (HSCT). Levels of ferritin and TS were measured at days -7 and -4 , respectively, in 25 patients who underwent HSCT after CY/TBI. The group consisted of 20 men and five women with a median age of 40 years. Fifteen patients were autotransplanted and 10 allografted. Nine of them had a diagnosis of AL, six of CML and 10 of lymphoma. Thirteen of them were in early and 12 in advanced status of disease. VHL of ferritin and TS $\geq 100\%$ were associated with a decreased OS ($P = 0.001$ and $P = 0.006$, respectively) and an increased TRM ($P = 0.003$ and $P = 0.004$, respectively) in univariate survival analysis. Both variables remained significant at multivariate analysis for OS ($P = 0.03$ and 0.02 , respectively) and TS was an independent factor for TRM ($P = 0.01$). Ferritin was very close to achieving statistical significance for TRM ($P = 0.06$) in multivariate analysis. In conclusion, VHL of ferritin and TS $\geq 100\%$ at conditioning are associated with an increase in toxic deaths after transplant.

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Iron overload (IO) is associated with free radical production and tissue damage in different diseases such as genetic haemochromatosis and secondary IO.^{1,2} IO may be present among patients who undergo haematopoietic stem cell transplantation (HSCT) due to prior blood transfusions. Pre-transplant iron status may be evaluated measuring ferritin levels pre-conditioning; that is a time when ferritin is

still neither increased as an acute phase reactant nor influenced by liver toxicity secondary to chemo-radiotherapy. Moreover, transferrin saturation (TS) increases during chemo-radiotherapy, often reaching indexes of over 80% and producing non-transferrin-bound iron (NTBI).^{3,4} TS might inform us about the potentially toxic effect of free iron, without any protein control, produced during conditioning. It is likely that an IO status pre-conditioning with an elevated TS during this procedure for HSCT may increase the toxic effects of chemo-radiotherapy.⁵ This could cause an increase in transplant-related mortality (TRM) and a subsequent decrease in overall survival (OS). The aim of this study was to determine whether a very high level of ferritin pre-conditioning and/or a full TS during CY-TBI were independent risk factors for TRM and OS.

Patients and methods

Twenty-five consecutive patients who underwent HSCT with CY/TBI were prospectively enrolled in the study. All patients were conditioned with cyclophosphamide 60 mg/kg once daily i.v. on days -6 and -5 (total dose 120 mg/kg) and TBI 6×2 Gy from days -3 to -1 . GVHD prophylaxis in allogeneic HSCT consisted of i.v. cyclosporin and a short course of methotrexate. Ferritin level and TS were measured at days -7 (pre-conditioning) and -4 (during conditioning), respectively, by commercial immunoassays. We considered very high levels (VHL) of ferritin and TS as $\geq 3000 \mu\text{g/l}$ and $\geq 100\%$, respectively. Additional variables registered for analysis were age, sex, baseline disease, status of disease (early vs advanced in cases of ≥ 2 CR or ≥ 2 PR and refractory disease), stem cell source (peripheral blood vs bone marrow), transplant type (allo vs auto) and number of blood units transfused prior to transplant. Differences between categorical variables were measured by the chi-square test, and differences between means in continuous variables with the Mann-Whitney U test. Kaplan–Meier and log-rank univariate comparisons were used to evaluate OS and TRM in univariate survival analysis. Cox regression was used in survival multivariate analysis. Results were considered as statistically significant when P values were less than 0.05.

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Results

The clinical characteristics of the 25 patients are summarized in Table 1. The source of the graft was peripheral blood in 11 patients and bone marrow in 14. No statistically significant differences in distribution of clinical parameters were found between patients with and without IO.

Four patients presented a VHL of ferritin prior to the conditioning regimen, and 11 a high TS during the procedure. The levels of ferritin correlated with the number of previous red cell transfusions ($P = 0.002$), but a statistical relation was not observed between TS and the number of previous transfusions or between TS and ferritin levels. VHL of ferritin at day -7 and $TS \geq 100\%$ at day -4 were associated with a decreased OS ($P = 0.001$ and $P = 0.006$, respectively) and an increased TRM ($P = 0.003$ and $P = 0.004$, respectively) in univariate survival analysis. An additive effect of both variables can be seen in Figure 1. The median time from HSCT to toxic death was 2.8 months (range 0.3–34) in the IO group (with at least one increased iron parameter) and 38 months (range 0.4–47) in the remaining patients.

Both variables remained significant at multivariate analysis for OS (RR 4.7, CI 95% 1.2–18.6, $P = 0.03$ and RR 3.5, CI 95% 1.2–10.5, $P = 0.02$, respectively), and TS at day -4 was also an independent prognostic factor for TRM (RR 6.7, CI 95% 1.5–29.9, $P = 0.01$). Moreover, ferritin at day -7 was very close to achieving statistical significance (RR 5, CI 95% 0.9–26.8, $P = 0.06$). This increase of TRM was related with a high infectious mortality. Seventy-five % of patients died with serious infection in the VHL of ferritin group at day -7 vs only 19% in the other group ($P = 0.05$). The same phenomenon reproduced with high TS at day -4 (55% vs 7%, $P = 0.01$). Among allo-transplanted patients, 2/6 developed acute GVHD grade III–IV in the IO group, and 1/4 in the non-IO patients.

Discussion

The first requirement for observing the pathological consequences of IO is to have patients with this metabolic disturbance. Some previous literature anticipated that IO may be usual in bone marrow recipients, and probably more frequent in those patients who died between 50 and 100 days after transplant.⁶ In the 25 patient group the prevalence of severe iron disturbances was very high, because four patients had a basal ferritin level above 3000 $\mu\text{g/l}$ and 11 a $TS \geq 100\%$ during conditioning. The median number of packed red cells transfused prior to transplant in these 25 patients was 8 (range 0–44), and a positive correlation existed between the number of packed red cells transfused and pre-transplant ferritin levels. Nevertheless, TS during conditioning did not correlate with prior transfusions or with ferritin levels and high TS seems to have a different origin and probably could measure other aspects of iron toxicity. TS increase has three possible explanations: a decrease in transferrin due to hepatic toxicity, stored iron leaking from injured liver to blood and, finally, a suppression of erythropoietic activity during treatment.³ It has recently been demonstrated⁴ that patients who underwent HSCT and were conditioned with CY-TBI experienced a marked increase in TS with a peak on day -4. According to the same authors, non-transferrin-bound iron (NTBI) was detected in almost all patients when TS exceeded 80%. Although we did not directly measure NTBI in our patients, it is likely that all with more than 100% TS had NTBI, and pathological consequences of high TS could be mediated by the presence of free iron in tissues.

In univariate survival analysis, VHL of ferritin and TS dramatically increase TRM and decrease OS, and these effects are mainly due to infectious mortality. Multivariate analysis maintains the significance of both variables for OS and TRM ($P = 0.06$ for ferritin and TRM). It is especially

Table 1 Clinical characteristics of patients

	Without IO ^a	With IO ^a	All patients
No. patients	13	12	25
Age ($P = 0.4$) median (range)	38 (18–56)	42 (17–59)	40 (17–59)
Gender ($P = 0.3$)			
Male	9	11	20
Female	4	1	5
Underlying disease ($P = 0.7$)			
Acute leukaemia	4	5	9
Chronic myeloid leukaemia	4	2	6
Malignant lymphoma	5	5	10
Disease status ($P = 0.7$)			
1st CR	2	3	5
2nd CR	1	3	4
1st PR	3	2	5
2nd PR	3	0	3
Non-treated 1st relapse	2	1	3
Refractory disease	2	3	5
Time from diagnosis to HSCT (in months) median (range)	11 (5–76)	12 (3–50)	12 (3–76)
Transplant type ($P = 0.4$)			
Autotransplants	9	6	15
Allotransplants	4	6	10

^aIO refers to patients with at least one increased iron parameter (ferritin level $\geq 3000 \mu\text{g/l}$ at day -7 or $TS \geq 100\%$ on day -4). Statistical differences between groups were not significant in any case.

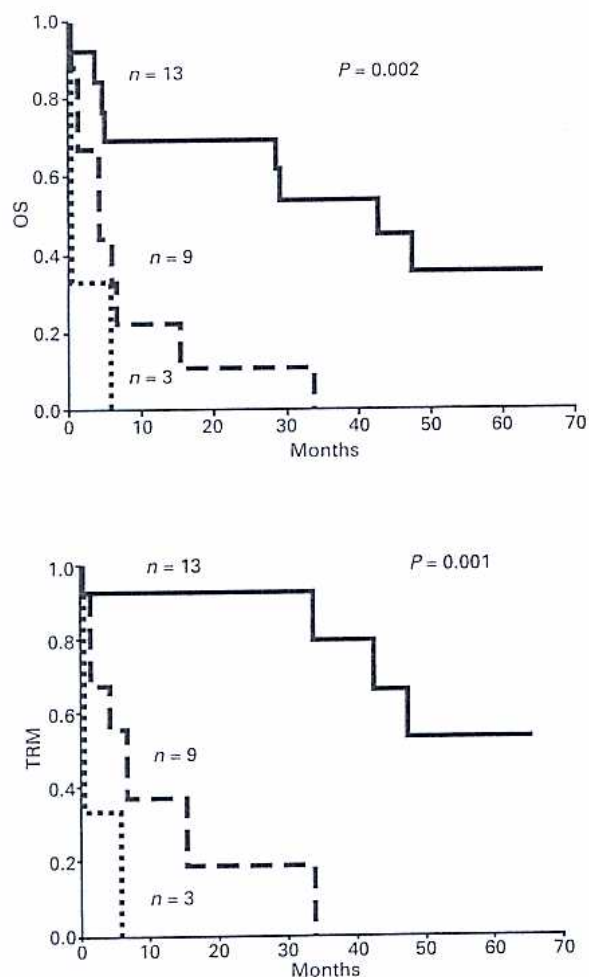


Figure 1 Kaplan-Meier estimates of overall survival (above) and TRM (below) in 25 patients after progenitor cell transplantation according to iron overload parameters. Continuous line represents patients without significantly altered iron parameters, segmented line, patients with only one altered parameter (ferritin level $\geq 3000 \mu\text{g/l}$ at day -7 or TS $\geq 100\%$ on day -4), and plotted line represents patients with both altered parameters.

interesting that the multivariate analysis was controlled by previous blood transfusions and other relevant clinical characteristics of patients. This demonstrates that these results are not simply related to a more severe disease or more severe toxicity. Ferritin and TS act as independent predictive factors influencing TRM and OS.

Iron in excess acts as a free radical catalyst of Fenton's reaction,⁷ causing toxicity and tissue damage. Mucositis and liver injury are common after HSCT and can be partly mediated by NTBI during cytotoxic chemoradiotherapy, as has been observed in children undergoing chemotherapy for ALL.⁸ In fact, IO has been identified as a cause of serious liver toxicity in the bone marrow transplant setting.⁹ Hyperferraemia can predispose to bacterial and fungal infections.¹⁰ Increased TS and ferritin are proven risk factors for the development of systemic fungal infections in patients with haematological malignancies,¹¹ and, accordingly, patients submitted to liver transplant caused by haemo-

chromatosis are at a greater risk of dying from fungal infections than patients without iron overload submitted to the same transplant type.¹² An increase in some late fungal infections, specially mucormycosis, have been reported in iron loaded patients after HSCT.¹³

Further studies to confirm our findings are mandatory. If these results are reproduced, pre-transplant strategies to decrease iron overload¹⁴ could be proposed to decrease TRM and improve survival.

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