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Frequent severe liver iron overload after stem cell transplantation and its possible association with invasive aspergillosis

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Summary:

Iron overload is associated with free radical generation and tissue damage. Our main objective was to ascertain the frequency and severity of iron overload in a group of 59 patients who died after conventional-intensity autologous ($n = 24$) or allogeneic ($n = 35$) haematopoietic stem cell transplantation (HSCT). A second objective was to investigate associations between liver-iron concentration and causes of transplant-related mortality. The median age was 41 years (range, 19–66), 41 were males and 18 females. In total, 26 patients had acute leukaemia or MDS, 10 CML, 17 lymphoma, four myeloma and two aplastic anaemia. The median hepatic iron concentration (HIC) was 138 μmol/g dry weight (7.7 mg/g; range 31–631 μmol/g). In total, 4/32 (12%) patients with HIC $<150\,\mu$mol/g and 10/27 (37%) with hepatic iron $\geqslant 150\,\mu$mol/g showed invasive aspergillosis at autopsy ($P = 0.035$). This was significant in multivariate analysis (RR 9.0; 95% CI 1.6–50.3, $P = 0.012$). In conclusion, severe iron overload is frequent in patients who die following HSCT and is associated with invasive aspergillosis.
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The consequences of iron overload during haematopoietic stem cell transplantation (HSCT) have been studied in diseases classically associated with siderosis, such as thalassaemia.[1–2] Iron overload is not usually taken into account when the indication for HSCT is a malignancy. Nevertheless, secondary iron overload may be frequent in this setting as these patients have often been heavily transfused prior to HSCT. Free iron acts as a free radical catalyser, and may increase mucositis, vasculitis, and other toxic effects of the conditioning regimen.[3–4] These adverse events and the high availability of free iron might increase microbial growth and the probability of severe infections.[5] Indeed, iron is an essential element for all pathological microorganisms. It is therefore possible that iron overload may significantly increase transplant-related mortality (TRM) due to toxic death and lethal infections in immunocompromised patients.

The main objective of this work was to describe the distribution of liver iron concentration in 59 liver samples from consecutive post-mortem studies in patients who died after conventional-intensity HSCT. We also investigated possible associations between hepatic iron concentration (HIC) and causes of TRM confirmed in a revision of pathology findings at post mortem.

Materials and methods

We retrospectively studied all post-mortem examinations performed at the Sant Pau Hospital Pathology Department from July 1990 to December 2001 in patients who had undergone conventional-intensity HSCT. Clinical and necropsy records were analysed, and all microscopic pathological samples were reviewed by an independent pathologist (FJS). Special attention was paid to causes of TRM such as veno-occlusive disease of the liver (VOD), acute graft-versus-host disease (GVHD), and severe infections. In all cases, stainable liver iron was assessed from paraffinated liver samples using Tirmann–Schmelzer's technique,[6] and biochemical iron quantification was performed with colorimetric analysis.[7] Our 95% CI for iron liver concentration in necropsy with this method is $13 \pm 8\,\mu$mol/g dry weight (dw).[8]

We defined patients as in the early clinical stage when they were in first complete or partial remission, in contrast with more advanced stages. The criteria established by the EORTC-MSG were used to define invasive fungal infections.[9] Since all findings were by histology, a definite diagnosis was made in each case. The presence of tissue-invasive branched septated hyphae or yeasts with or without pseudohypae in lung and/or other organs plus a positive pre- or post-mortem culture was defined as definite invasive aspergillosis and candidiasis, respectively. CMV disease was defined by the presence of CMV-like

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inclusions by histology plus a positive culture and/or immunohistochemistry.[10] Acute and chronic GVHD were staged by established criteria[11,12] and histological evidence at autopsy. Other infections were defined by standard histological and microbiological findings at autopsy. All potentially lethal relevant findings at autopsy were considered as contributory to death (GVHD, invasive aspergillosis, VOD, etc).

Statistical methods

Descriptive statistic parameters were used to determine liver iron concentration in this series of patients. A lineal regression was calculated between liver iron concentrations measured by pathological and biochemical methods. Associations between different liver iron levels and categorical variables (causes of TRM) were studied by the χ^2 test. If an association between liver iron concentration and a cause of TRM was detected, a logistic forward stepwise regression model was used to ascertain whether the liver iron concentration had an independent statistical value to predict the cause of TRM. The results were considered statistically significant at $P<0.05$.

Results

Liver samples obtained at post-mortem study in 59 patients previously submitted to HSCT were revised. Clinical data were obtained in all cases from our transplant database. Baseline data in this series prior to transplant are shown in Table 1.

Toxicity

Six patients did not engraft, and one patient experienced secondary graft failure. VOD was diagnosed clinically in four patients. In total, 17 developed grade III–IV acute GVHD in the first 100 days post-transplant, and six patients developed extensive chronic GVHD after this time. In total, 34 patients had evidence of infection at necropsy: 11 bacterial, 21 viral (17 CMV), and 20 fungal (six *Candida* and 14 *Aspergillus*). In total, 23 patients relapsed before death. The overall median post-transplant survival in this series was 5 months (95% CI 3–6).

Liver iron concentrations

The correlation between pathological and biochemical methods to measure liver iron was good ($R^2=0.8$, $P<0.001$). The median liver iron concentration in these patients was 138 μmol/g (7.7 mg/g) dw (range 31–631 μmol/g dw). The concentration was $>100\,\mu$mol/g (5.6 mg/g) dw in 41 (70 %), and $>200\,\mu$mol/g (11.2 mg/g) dw in 17 (29%). The hepatic iron index was over 1.9 in 49 patients (83%). Differences in iron load between patients after allo or autotransplant were not significant; median liver iron concentration 146 μmol/g (8.2 mg/g) dw and 133 μmol/g (7.4 mg/g) dw, respectively ($P=0.9$).

Table 1 Baseline characteristics of the 59 patients prior to transplant

Age	
Median (range)	41 (19–66)
Gender	
Male/female	41/18
Diagnosis	
Acute leukaemia	23
Myelodysplastic syndromes	3
Chronic myeloid leukaemia	10
Lymphoma	17
Multiple myeloma	4
Aplasia	2
Stage	
Early	31 (52%)
Advanced	28 (48%)
Type of transplant	
Autologous	24 (41%)
Allogeneic	35 (59%)
Source of stem cells	
Bone marrow	31 (52%)
Peripheral blood	28 (48%)
Conditioning	
With TBI	36 (64%)
Without TBI	23 (36%)
GVHD prevention (allo only)	
Cyclosporine and methotrexate	24 (69%)
Cyclosporine and prednisone	4 (11%)
Cyclosporine only	7 (20%)

Relation with causes of TRM

Possible associations between transplant events and a high liver iron concentration (defined as $\geqslant 150\,\mu$mol/g or 8.4 mg/g. dw) were investigated. Univariate analyses showed no relationship between liver iron and VOD, GVHD (acute and chronic) and death related with bacterial or viral infections. TRM at 6 months and median survival of patients with liver iron concentrations above and under 150 μmol/g dw was also similar. No relationship was found between *Candida* infection and liver iron content. Nevertheless, a statistically significant relationship was found between invasive *Aspergillus* infection and a high liver iron concentration (Table 2). Other variables related with *Aspergillus* infection were the presence of acute GVHD grade III–IV ($P=0.046$) and concomitant CMV infection ($P=0.015$). No relationship was found between *Aspergillus* infection and graft failure or the duration of disease. We also analysed the relationship between severe iron overload and *Aspergillus* infection in each transplant category. In the allotransplanted group, 6/17 patients (35%) with severe iron overload had *Aspergillus* infection at necropsy, as compared to 4/18 patients with a lower iron level (22%, $P=$NS). In the autotransplant group, 4/10 patients (40%) with a high iron concentration developed *Aspergillus* infection, while none of the 14 patients with a lower iron level presented this infection (0%, $P=0.02$). Some clinical

Table 2 Relationships between HIC and causes of TRM

	HIC < 150 μmol/g dw	*HIC ⩾ 150 μmol/g dw*	*P*
VOD	4/32 (12%)	0/27 (0%)	0.1
aGVHD III–IV	10/18 (55%)	9/17 (53%)	1
Ext cGVHD	3/8 (37%)	3/7 (43%)	1
Bacterial infection	8/32 (25%)	3/27 (11%)	0.2
Viral infection	13/32 (41%)	8/27 (30%)	0.4
CMV infection	11/32 (34%)	6/27 (22%)	0.4
Candida infection	3/32 (9%)	3/27 (11%)	1
Aspergillus infection	**4/32 (12%)**	**10/27 (37%)**	**0.035**
TRM 6 months	15/32 (47%)	14/27 (52%)	0.8

Bold represents statistically significant.

Table 4 Multiple logistic regression analysis for independent and significantly related variables with the presence of *Aspergillus* sp infection in post-mortem studies

	Slope(B)	*P*	*Exp(B)*	*95% CI Exp(B)*
CMV infection	2.4	0.005	11.3	2.1–61.7
HIC > 150 μmol/g dw	2.2	0.012	9	1.6–50.3

Other variables included in the model were age, gender, diagnosis, stage (early or advanced), type of transplant, and presence of graft failure or aGVHD (grades III–IV *vs* others). $N = 59$.

Table 3 Clinical characteristics of patients with invasive aspergillosis at death and high HIC (⩾150 μmol/g dw)

	Gender	*Age*	*Disease*	*Transplant*	*Stage*	*aGVHD*	*cGVHD*	*CMV*	*Engraftment*	*Graft failure*
1	Male	61	NHL	Allo	Early	III	No	Yes	Yes	No
2	Male	36	AML	Allo	Advanced	IV	No	Yes	Yes	No
3	Male	22	Aplastic anaemia	Allo	Early	III	Extensive	Yes	Yes	No
4	Male	24	ALL	Auto	Early	—	—	No	Yes	No
5	Male	45	HD	Auto	Advanced	—	—	No	Yes	Yes
6	Male	45	AML	Allo	Early	II	No	No	Yes	No
7	Male	20	ALL	Auto	Advanced	—	—	No	Yes	No
8	Male	66	AML	Auto	Early	—	—	No	Yes	No
9	Female	38	CML	Allo	Early	IV	No	No	Yes	No
10	Male	49	AML	Allo	Early	III	Extensive	Yes	Yes	No

characteristics of patients with high iron levels and invasive aspergillosis at death are shown in Table 3. Most of them had other relevant risk factors for aspergillosis such as acute GVHD or graft failure. Nevertheless, in the multivariant analysis adjusted for age, gender, diagnosis, stage, type of transplant, presence of graft failure and acute GVHD (grades III–IV *vs* others), only two variables remained significant to predict the appearance of *Aspergillus* infection; a liver iron concentration ⩾150 μmol/g dw and a concomitant CMV infection (Table 4).

Discussion

Iron overload at conditioning has been associated with an increase in toxic deaths after transplant.[13] Strasser *et al*[14] examined the hepatic iron overload in 10 consecutive patients who died between 50 and 100 days after transplant. The median liver iron concentration was 77 μmol/g dw (range 33–235) with a median HII of 3.85 (range 0.76–8.14). Iron overload is also common in paediatric bone marrow transplantation.[15] Our findings confirm these data, with a median HIC in our series of 138 μmol/g dw (range 31–631) and a median HII of 3.7 (range 0.6–15.3), with HII >1.9 (criteria of genetic hemochromatosis) in 83% of patients. Our 95% CI for iron liver in necropsy was slightly higher than that reported using other methods, but the very high iron levels detected cannot be explained by a methodological bias. The concordance between pathological and biochemical methods to measure liver iron was also high. Causes of iron overload in HSCT patients are probably diverse, although transfusions are likely to be the main cause.

While one may expect VOD to be related to iron overload since iron is fundamentally stored in liver,[16] the four patients with VOD had near normal iron stores. These data are too limited to allow any conclusions to be drawn on the role of iron in the pathogenesis of VOD.

Although iron is constantly present in human body fluids, the amount of free iron, which might be readily available to bacteria and fungi, is low under physiological conditions (about 10^{-18} M). Transferrin saturation increases during chemoradiotherapy, often reaching indexes of over 80% with the presence of nontransferrin-bound iron.[17,18] The coexistence of a high plasma iron availability with damage to the mucosal barrier could predispose to infectious events. Bacteria, fungi and protozoa have very active iron-uptake systems and can therefore multiply successfully *in vivo* in order to establish an infection.[19] A clinical example of this is the increased rate of zygomycosis among dialysis patients treated with desferrioxamine because iron bound to desferrioxamine is efficiently used as an iron source by some species of *zygomycetes*.[20] We saw an association between *Aspergillus* and iron overload, more clearly with autografts than with allografts. In allograft patients, other potentially more important variables such as acute GVHD, immunosuppression, CMV, and steroid treatment may mask any effect of iron.

How do we explain the iron overload relationship found with *Aspergillus* spp but not with other pathogens? Two experimental observations *in vitro* can help us to explain this interesting phenomenon. First, *Aspergillus fumigatus* is the only fungal pathogen able to thrive and grow in the presence of up to 80% human serum and up to 80% FBS. In addition, the levels of proteinase secretion in *A. fumigatus* (an important factor of pathogenicity, invasiveness and lethality) are very high when either human serum or FBS are present. It seems *A. fumigatus* is able to use iron in serum and that a combination of available iron and protein in serum permits abundant growth of the pathogen.[21] Second, the mechanism by which *A. fumigatus* obtains iron in serum-containing media has recently been revealed. Five siderophores were purified from *A. fumigatus* culture medium, and the two major siderophores were identified as triacetylfusarinine C and ferricrocin. Both molecules can remove iron from holotransferrin with an affinity comparable to that of ferrichrome. *A. fumigatus* survival in human serum involves siderophores-mediated removal of iron from transferrin. Proteolytic degradation of transferrin by proteinases may play a secondary role in iron acquisition.[22] Indeed, the deletion of *A. Nidulans* gene sidA completely blocks triacetylfusarinine C and ferricrocin biosynthesis, and siderophore-deficient strains are unable to grow.[23] Two experimental findings *in vivo* can be related with the iron-dependency of *Aspergillus:* the preventive effect of endotoxin against *Aspergillus* infection in mice,[24] and the delayed lethal response to *A. fumigatus* infection in sarcoma 180 tumour-bearing mice. These results may be mediated by a significant decrease in serum iron levels and an increase in unbound iron binding capacity in these mice as compared to nonmanipulated mice.[25] Iron is so essential for the growth of fungal hyphae that intracranial aspergillomas can be identified by MRI with a low signal on T2-weighted images, corresponding to iron accumulation.[26] Interestingly, previous clinical papers have related iron overload and infection in immuno-deficient patients, and the infection was always produced by *Aspergillus* or *Mucor*.[27–29] In our series, *Aspergillus* infection was not related with neutropenia or acute GVHD, probably because 41% of cases were autologous transplants and the number of patients with long-standing neutropenia or severe acute GVHD was low. An association between CMV disease and fungal infections has been noted not only in solid organ transplant recipients[30,31] but also in recipients of HSCT.[32–34] The relationship between both pathogens remains obscure.

This work shows that severe iron overload is frequent in patients who die after HSCT, and for the first time, it reveals a relationship between severe iron overload and invasive aspergillosis. Nevertheless, the number of patients examined is low, and the results should be confirmed in studies in larger series. The cumulative incidence rate of invasive aspergillosis (about 14%) after myeloablative and nonmyeloablative conditioning has increased in recent years[34] and is one of the main causes of death in transplanted patients. If these results are reproduced with prospective data, pre-transplant manoeuvres to decrease iron overload[35] or transferrin saturation with human transferrin infusions during conditioning[36] would be proposed to lower TRM and improve survival.

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