Quantification by magnetic resonance imaging and liver consequences of post-transfusional iron overload alone in long-term survivors after allogeneic hematopoietic stem cell transplantation

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ABSTRACT

We quantified and studied the impact of post transfusional iron overload alone in post allogeneic HSCT. Median number of RBCs was 18. Ferritin was 532 µg/L. Liver iron content (LIC) was 117 µmoles/gdw. Correlation RBCs and ferritin was (r=0.81); RBCs and LIC was (r=0.84). The high ferritin group differed from normal ferritin group in terms of RBCs transfused ($p<10^{-3}$), ALT ($p<0.009$). But occurrence of liver dysfunction was not significant. Magnitude of iron overload correlates closely to the number of RBCs and is quantified by MRI. Impact on liver dysfunction is moderate in absence of co-morbidity.

Key words: manetic resonance imaging, iron overlaod, allogeneic hematopoietic stem cell transplantation

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The consequences of iron overload on liver function in multitransfused thallassemic patients are well known[1] but little data are available in patients receiving blood transfusions for others reasons. Post transfusional iron overload is potentially damaging and has already been suggested in various acquired chronic anemia.[2,3] Previously healthy patients who received a known quantity of red blood cells over a limited period and were followed up over a long period, represent a good theoretical model to study long-term effects of iron overload induced by transfusion only. Long-term survivors after allogeneic HSCT theoretically correspond to the above criteria iron overload has been reported in up to 88% after allogeneic HSCT.[4-6] However, estimation of iron overload has mainly been based on ferritin only. However, the many confounding factors in post transplant, such as infection, inflammation, drug toxicity, chronic graft-versus-host disease (GVHD), veno-occlusive disease result in frequent ferritin overestimation.[6,7] Furthermore, the high prevalence of hepatitis C in post transplant makes measuring the consequences of iron overload on liver dysfunction unreliable. The ability to evaluate liver iron content (LIC) by MRI without biopsy[8] and the absence of previous treatment of iron overload in our patients afforded us a unique opportunity to measure and study consequences of iron overload in a large number of patients transplanted at a single institution. Patient selection criteria attempted to avoid the many confounding factors both in the evaluation of iron overload and its consequences on liver dysfunction. We quantified iron overload and studied the relationship between the amount of red blood cell units, LIC estimated by MRI, hepatocellular injury and clinical consequences.

Design and Methods

This prospective study was carried out for a 21 month period from June 2002 to March 2004. All adult patients, surviving for at least four years after allogeneic HSCT, followed up at our center were included. We systematically looked for oral drug use, confirmation of alcoholism according to the CAGE questionnaire,[9] clinical presence of any form of GVH, documented history of bleeding or transfusion in the previous year, recording of the total number RBCs administered before and during transplant procedure (from blood delivery software or from the medical files

before 1990). At enrollment biologic evaluation of all patients was carried out according to routine procedures. This included inflammation status (sedimentation rate, C reactive protein, albumin), hepatitis B and C status (serology), iron status (ferritin and siderophilline saturation), liver function (AST, ALT, total bilirubin, gammaglutamyl transpeptidase) and glycemia. In patients with serum ferritin (above normal value), a quantitative measurement of LIC by T2* MRI using gradient echo sequences and signal intensity ratio was performed as previously described.[10] An evaluation of HFE (C282Y) status at pre-transplant on available frozen DNA was made. Exclusion criteria were: hepatitis C or active hepatitis B, alcoholism (at least one positive response according to CAGE questionnaire), patients continuing immunosuppressive therapy or hepatotoxic drug administration, patients with clinical GVH or prior histologically documented chronic graft versus host disease or veno-occlusive disease, patients with active documented bleeding requiring blood transfusion after the one year period following transplant, patients with inflammation (sedimentation rate of more than 30 and/or C Reactive Protein more than >5 mg/L), patients who developed a neoplastic disease or relapsed during the 21 month study period.

A specific clinical examination to determine the effects of iron overload (cardiomyopathy, arythmia) was performed. Phlebotomies were proposed to all patients with serum ferritin above normal value and iron overload confirmed by MRI. All statistics were managed by SPSS software.

Results and Discussion

Patients

A hundred and four adult patients were enrolled. Thirty-nine were excluded for various reasons: GVH n=11, hepatitis n=3, alcoholism n=1, hepatotoxic drug n=4, active bleeding requiring blood support after the one year period following the transplant (n=2 one gastric adenocarcinoma, one gastric ulcer), secondary neoplastic disease or relapse during the 21 month evaluation period (n=11), chronic inflammation (n=7). Clinical characteristics of the 65 patients included in the study are shown in Table 1.

Iron overload

The median of ferritin was 532 µg/L (42-4023). Twenty-seven patients had a ferritin value within normal range. Thirty-eight (58%) patients had a ferritin value above normal. Among these patients, MRI evalution of LIC was performed in 32 cases (contraindication in 3 cases and refusal in 3 cases). LIC was above normal 36 µmole/g dry weight hepatic tissue (µmoles/gdw) in 31/32 cases. One man had a MRI value within normal range with a ferritin value at 390 µg/L. The median of LIC was 117 µmoles/gdw (mean 126) (30->300). Four patients had transferrin saturation above normal value. No patients were homozygous for HFE mutations. HFE analysis was performed on DNA skin in only 3/36 cases in which DNA frozen before transplant was unavailable. Data on blood units transfused were obtained from blood delivery software in 38 cases and from medical files in 22 cases. The median number of RBCs received was 18 (range 0-77) which corresponds to a median of 3.5 g of iron per patient (range 0-15.4). The median number of RBCs in patients with acute leukemia was 23.5 (4-70) and in patients without leukemia was 13 (0-77) (p=0.002).

Table 1. Main clinical characteristics of patients.

Age median at transplant (year) (range)	35.9 (18-52)
Sex (Male/female)	34/31
Underlying disease	
Acute leukemia	24
Chronic myeloid leukemia	28
Others	13
Lymphoma	6
Myeloma	1
Myelodysplastic syndrome	5
Other myeloproliferative disease	1
Follow-up (years) median (range)	8.8 (4.2-17.1)

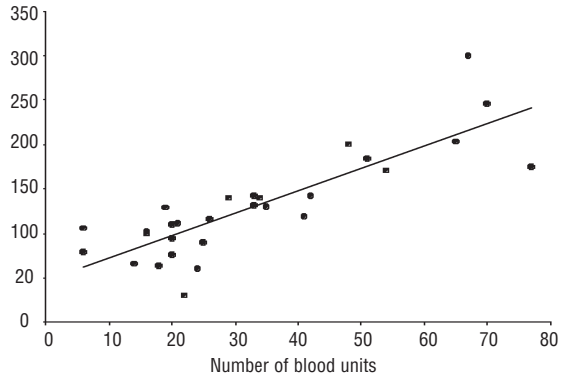

Figure 1. Correlation between number of blood units transfused and liver iron concentration (LIC) estimated by MRI.

Correlation study

There was a significant correlation between the number of RBCs transfused and ferritin value (r=0.81) ($p<0.0001$) and between the number of RBCs and the LIC estimated by MRI (r=0.84) ($p<0.0001$) (Figure 1). The correlation was significant between the ferritin value and the LIC estimated by MRI (r=0.55) $p=0.001$. There was no effect of age, date of transplant, or HFE status on ferritin or on LIC estimated by MRI. There were significantly more patients with acute leukemia in the high ferritin group (56.2%) than in the normal ferritin group (14.8%) ($p=0.002$).

Table 2. Impact of number of RBCs (+- 20) on ferritin, LIC by MRI, and occurrence of liver dysfunction.

		N	*Mean*	*Median*	*IC 95*	*p*
Ferritin ng/mL	RBC ≤20	36	360	254	2536467	$< 10^{-3}$ (t-test)
	RBC >20	24	1356	1097	982-1730	
AST UI/L	RBC ≤20	36	25	23		ns (Mann-Whitney)
	RBC >20	24	32.9	25.5		
ALT UI/L	RBC ≤20	36	28	25		ns (Mann-Whitney)
	RBC >20	24	42.7	26.5		
LIC µmoL/gdw	RBC ≤20	9	92	94	75-109	0.013 (t-test)
	RBC > 20	19	149	140	119-179	

Clinical and biological consequences

No patients had clinical cardiopathy or were arythmic. The high ferritin group (n=38) differed significantly from the normal ferreting group, particularly with regards to the number of RBCs ($p<10^{-3}$), the level of AST (p<0.017) and ALT (p<0.009). There was no significant difference in glycemia. However, the risk of occurrence of liver dysfunction (AST or ALT above normal value) demonstrated a different trend from the normal ferritin (8/27, 29.6%) and high ferritin (19/38, 50%) groups. But was not significant (χ^2 not significant). Also, in the group which had received more than 20 RBCs there was a slight, statistically insignificant trend in the risk of occurrence of liver dysfunction compared with the group which had received less than 20 RBCs (n=36). However, there was a surprisingly significant difference between these two groups in terms of ferritin and LIC results by MRI (Table 2).

Evolution after phlebotomies

Venesections were performed on 29/31 patients. Nineteen had ALT and /or ALT above normal value. Ten out of the 16 evaluable patients showed normalized hepatic biology after phlebotomies (well tolerated in all cases). Ferritin was normalized in 24 /28 evaluable cases. A clear persistent iron overload is encountered late after transplant in at least 58% of cases. This result is smaller than in other series6 mainly due to our selective approach that systematically excluded patients with possible confounding factors which could overestimate ferritin. This approach was necessary to allow us to study the impact of iron overload induced by transfusion alone. A spontaneous decrease in iron overload has been described early in post transplant[11] while our results confirm a clear persistence of iron overload very late after transplant. The magnitude of this iron overload estimated by ferritin and/or MRI closely correlates to the number of RBCs received. Until now, this correlation had not been reported in post transplant patients. MRI can quantify iron[8] but it is not standardized. The correlation is satisfactory with our MRI technique. However recent MRI techniques are more precise. They are less influenced by liver fibrosis[12] and better adapted to high liver iron overload concentrations.[12,13] The correlation with ferritin is good as long as other individual co-factors are eliminated. Indeed it has been recently demonstrated that the change in serum ferritin over time parallels changes in LIC.[14] In late post transplant, iron overload seems to be induced mainly by transfusion only and our data confirm that magnitude of iron overload is related to the underlying disease requiring more transfusion. The impact of transfusion alone on liver dysfunction is moderate in this group of patients without other co-factors of hepatotoxicity. These co-factors are frequently encountered in post-transplant and have previously been shown to seriously compromise liver function and fibrogenesis.[15,16] They could be involved in a particular evolution of hepatitis C in post transplant[17] which is responsible for the large majority of subsequent cirrhosis.[18] Our selection criteria could underestimate the clinical impact of iron overload. In our opinion, however, this moderate impact should not detract from the need for preventative phlebotomies given the frequency of co-factors in post transplant and the proven efficacy and feasibility of these procedures.

Authors' Contributions

CR designed the study, wrote the paper and followed the clinical aspect of iron overload for all the patients; OE performed the MRI studies in all patients; BH did the statistical analysis; PM recorded the biochemical data; PR recorded the transfusion related data; MPN, IY-A, and JPJ included patients.

Conflict of Interest

The authors reported no potential conflicts of interest.

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