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Early clinical impact of iron overload in stem cell transplantation. A prospective study
Altes · Remacha · Sarda · Baiget · Sureda · Martino · Briones · Brunet ·
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89	Abstract	Toxic-infectious complications may be related with iron toxicity after a stem cell transplant (SCT). Eighty one patients who underwent SCT were prospectively evaluated over 3 months for mucositis, bacteraemia and febrile days. Pre-SCT transferrin saturation (TS), ferritin level and the number of days with TS \geq 80% after transplant were determined. A ferritin level $>1,500 \mu\text{g/l}$ predicted the appearance of severe mucositis, bacteraemia and days with fever in univariate ($P = 0.03$, $P = 0.03$ and $P = 0.03$) and multivariate analysis ($P = 0.03$, $P = 0.006$ and $P = 0.002$). Nevertheless, further statistical studies revealed that the predictive value of pre-SCT ferritin levels was restricted to AUTO-transplanted patients in both univariate ($P = 0.05$, $P = 0.05$ and $P < 0.001$) and multivariate ($P = 0.03$, $P = 0.05$ and $P < 0.001$) analysis, in contrast with the ALLO-transplanted group where this variable did not reach statistical significance. In conclusion, iron burden seems to influence the appearance of toxic-infectious complications during the first 3 months after transplant in AUTO-transplanted patients.	
90	Keywords separated by ' - '	Iron overload - Stem cell transplantation - Mucositis - Bacteraemia - Febrile days	
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3 ORIGINAL ARTICLE

4 **Early clinical impact of iron overload in stem cell**
5 **transplantation. A prospective study**

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14 **Abstract** Toxic-infectious complications may be related with
15 iron toxicity after a stem cell transplant (SCT). Eighty one
16 patients who underwent SCT were prospectively evaluated
17 over 3 months for mucositis, bacteraemia and febrile days.
18 Pre-SCT transferrin saturation (TS), ferritin level and the
19 number of days with TS≥80% after transplant were deter-
20 mined. A ferritin level >1,500 µg/l predicted the appearance
21 of severe mucositis, bacteraemia and days with fever in
22 univariate ($P=0.03$, $P=0.03$ and $P=0.03$) and multivariate
23 analysis ($P=0.03$, $P=0.006$ and $P=0.002$). Nevertheless,
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26 patients in both univariate ($P=0.05$, $P=0.05$ and $P<0.001$)
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29 variable did not reach statistical significance. In conclusion,
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31 infectious complications during the first 3 months after
32 transplant in AUTO-transplanted patients.

Keywords Iron overload · Stem cell transplantation · 33
Mucositis · Bacteraemia · Febrile days 34

Introduction 35

Chemoradiotherapy conditioning for stem cell transplantation 36
(SCT) causes toxicity and immunosuppression leading to 37
organ damage and infectious diseases, fundamentally within 38
first 3 months of the procedure. Iron overload (IO) is a frequent 39
condition in SCT [2, 17], and it can increase toxic and 40
infectious events. Free iron acts as a free radical catalyser and 41
may aggravate mucositis, vasculitis and other toxic effects of 42
the conditioning regimen [7, 11]. Moreover, the high 43
availability of free iron might increase microbial growth and 44
the probability of severe infections. Indeed, iron is an 45
essential element for all pathological microorganisms [6]. 46

An association between IO and infectious SCT compli- 47
cations was detected in previous studies [1, 2, 15]. In the 48
present work, we prospectively evaluated the impact of 49
biochemical IO on the appearance of toxic and infectious 50
events (severe mucositis, bacteraemia and days with fever) 51
in the first 3 months after SCT. 52

Materials and methods 53

Patients who underwent a peripheral blood SCT as 54
consolidation therapy were prospectively enrolled in the 55
study over a period of 1.5 years. Allogeneic non-related 56
transplants were excluded. All patients were in complete 57
remission (CR) or presented an objective response (OR) in 58
case of myelomas. ECOG was always ≤2. Finally, 81 59
patients who fitted the inclusion and exclusion criteria were 60
selected. All patients gave written informed consent, and 61

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62 the study was approved by the Institutional Ethics Com- 89
 63 mittee. The patients' characteristics are summarised in 90
 64 Table 1. 91

65 *Conditioning regimens* The conditioning regimens used 92
 66 were: cyclophosphamide—total body irradiation in 19 93
 67 cases, high dose cyclophosphamide, carmustine and etopo- 94
 68 side (CBV) in 10 cases, high-dose carmustine, etoposide, 95
 69 citarabine and melphalan (BEAM) in 10 cases, high-dose 96
 70 melphalan in 19 cases and a fludarabine-based reduced 97
 71 intensity conditioning (RIC) [14] in 23 cases. Peripheral 98
 72 blood stem cells were infused on day 0.

73 *Blood parameters related with iron metabolism (independ- 99
 74 ent study variables)* Transferrin saturation (TS) and ferritin 100
 75 were serially measured in each patient. A first sample was 101
 76 extracted pre-SCT, and the others were taken twice weekly 102
 77 from day 0 until neutrophil recovery [absolute neutrophil 103
 78 count (ANC) ≥ 500/μl]. In all patients, the number of days 104
 79 with TS > 80% from day 0 were calculated and recorded for 105
 80 analysis. The HFE genotype was determined in all cases by a
 81 previously published method [3].

82 *Variables related with infection or toxicity (dependent 107
 83 variables of study)* We recorded three post-SCT clinical 108
 84 variables related with infection or toxicity in the first 109
 85 90 days after transplant: 110

- 86 – Mucositis: This was assessed by specifically trained 111
 87 research nurses. Mucositis data, including stomatitis, 112
 88 dysphagia-esophagitis and colitis, were reviewed and 113
 114

t1.1 **Table 1** Clinical characteristics of patients

t1.2 Clinical characteristics	
t1.3 Sex	
t1.4 Men	43 (53%)
t1.5 Women	38 (47%)
t1.6 Median age	54 years (range 19–70)
t1.7 Disease	
t1.8 Leukaemia and transformed MDS	29 (36%)
t1.9 Multiple myeloma	28 (35%)
t1.10 Lymphoma	24 (29%)
t1.11 Disease status	
t1.12 1st CR–OR	33 (40%)
t1.13 ≥2nd CR–OR	48 (60%)
t1.14 SCT type	
t1.15 Autologous	50 (62%)
t1.16 Allo conventional	8 (10%)
t1.17 Allo RIC–SCT	23 (28%)
t1.18 TBI use	
t1.19 Yes	19 (23%)
t1.20 Not	62 (77%)
t1.21 CD 34+ × 10 ⁶ /Kg cells infused	5.81; CI 95% 4.1–7.5
t1.22 Median days to achieve ANC ≥ 500/μl	14 (range 9–25)

graded 0 to 4 as defined in the Common Toxicity 89
 Criteria from the National Cancer Institute CTC 90
 Version 2.0, April 30, 1999 ([http://ctep.cancer.gov/](http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf) 91
[forms/CTCv20_4-30-992.pdf](http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf)). 92

- Presentation of bacteraemia. Patients with positive 93
 blood cultures for skin contaminant pathogens were 94
 included if at least two blood cultures yielded the same 95
 organism with the same antibiogram. 96
- Cumulative number of febrile days during transplant 97
 (axillary temperature > 38°C). 98

Additional clinical variables Eight clinical variables were 99
 recorded to be included as modifier factors in multivariate 100
 analysis: sex, age, baseline disease, status of disease (1st 101
 CR–OR vs ≥ 2nd CR–OR), SCT type (autologous, 102
 conventional-allogeneic and RIC allogeneic SCT), TBI 103
 use, number of CD 34+ × 10⁶/Kg cells infused and number 104
 of days until neutrophil recovery (ANC ≥ 500/μl). 105

Statistical methods 106

Differences between categorical variables were measured 107
 by the chi-square test, and differences between means in the 108
 continuous variables were calculated with the Student's *t* 109
 test. Logistic regression was used to evaluate variables 110
 related to the appearance of grade III–IV mucositis and 111
 positive bacteraemia and a multiple regression in the case 112
 of the number of febrile days. Results were assessed as 113
 statistically significant when *P* values were less than 0.05. 114

Results 115

Iron parameters in the 81 patients are summarised in Tables 2 116
 and 3. The 75 percentile of independent variables (TS, serum 117
 ferritin and days with TS > 80%) was selected as threshold 118
 value to study the statistical relationships between these 119
 variables and toxic-infectious events (see Tables 2 and 3). 120

In the case of pre-SCT TS, 32% of patients with TS ≥ 121
 45% and 21% below suffered grade III–IV mucositis (*P*= 122

Table 2 Blood parameters re-
 lated with iron metabolism

HFE Genotype		t2.1
C282Y		t2.2
Wild type	76 (94%)	t2.3
Heterozygous	5 (6%)	t2.4
Homozygous	0 (0%)	t2.5
H63D		t2.6
Wild type	41 (51%)	t2.7
Heterozygous	36 (44%)	t2.8
Homozygous	4 (5%)	t2.9

t3.1 **Table 3** Blood parameters related with iron metabolism with 75 percentile of independent variables selected as threshold value

t3.2	Serum iron parameters			
t3.3	Mean	CI 95%	75 percentile threshold	
t3.4	Serum iron	17.05 µM/l	15.3–18.8	Non-defined
t3.5	TIBC	47.8 µM/l	45.9–49.6	Non-defined
t3.6	TS	37.3%	32.4–42%	≥45%
t3.7	Ferritin	954 µg/l	746–1163	≥1,500 µg/l
t3.8	Days with TS>80%	8.6 days	7.8–9.5	≥11 days

123 0.25). Bacteraemia was present in five patients (26%)
 124 above this level and in 14 (23%) below ($P=0.5$). The mean
 125 number of days with fever was very similar in both groups
 126 (4.2 vs 4.1, $P=0.9$).

127 Four of 23 patients that maintained a $TS \geq 80\%$ for
 128 ≥ 11 days from day 0 experienced grade III–IV mucositis
 129 (17%), as compared to 15/58 (26%) below this level ($P=$
 130 0.3). Percentages of patients with bacteraemia were similar
 131 in both groups (17 and 26% respectively, $P=0.3$), and both
 132 groups had a similar mean number of days with fever (3.5
 133 in front of 4.4, $P=0.9$).

134 Interestingly, patients with a ferritin level $\geq 1,500$ µg/l more
 135 frequently presented grade III–IV mucositis and episodes of
 136 bacteraemia, and they had more febrile days after transplant.
 137 Indeed, 8/19 patients above this threshold (42%) had severe
 138 mucositis and bacteraemia, as compared to 11/62 (18%) patients
 139 below ($P=0.03$). Microbiological results for all bacteraemic
 140 episodes are detailed in Table 4. The mean number of days
 141 with fever in patients above this ferritin threshold was 5.7,
 142 while it was 3.6 in the group below this level ($P=0.03$, CI
 143 95% of difference 0.18–3.9). When we compared the
 144 results from patients with ferritin levels below the 50
 145 percentile (0–849) with those from the third quartile (849–
 146 1,499 µg/l) and from the fourth ($\geq 1,500$ µg/l), we find that
 147 7/43 patients (16%) had mucositis and bacteraemia in the

t4.1 **Table 4** Microbiological results of the 19 bacteraemic episodes in accordance with ferritin level

t4.2	Ferritin level	<849 µg/l	Ferritin 849–1499	Ferritin \geq 1500 µg/l
t4.3	Number of patients	43	19	19
t4.4	Bacteraemic episodes	7 (17.7%)	4 (21%)	8 ^a (42.1%)
t4.5	<i>S. epidermidis</i>	4	2	3
t4.6	<i>P. aeruginosa</i>	1	2	2
t4.7	<i>E. coli</i>	1	0	0
t4.8	<i>S. viridans</i>	1	0	0
t4.9	<i>S. haemolyticus</i>	0	0	3
t4.10	<i>K. pneumoniae</i>	0	0	1
t4.11	<i>Fusobacterium</i>	0	0	1

t4.12 ^aIn two bacteraemic episodes two micro-organisms were found

148 first group, 4/19 (21%) in the second and 8/19 (42%) in the
 149 third (lineal association test $P=0.03$). Curiously, the three
 150 groups presented the same percentages of mucositis and
 151 bacteraemia but in different individuals in each group. The
 152 mean number of days with fever in both the first and
 153 second groups was 3.6, while it was 5.7 in the third group
 154 ($P>0.05$).

155 The results were similar in the multivariate analysis. After
 156 introducing the study variables and the additional clinical
 157 variables specified in **Materials and methods** into a logistic
 158 regression model, only ferritin $\geq 1,500$ µg/l maintained its
 159 value as an independent predictor of appearance of grade III–
 160 IV mucositis (RR 3.4, CI 95% 1.1–10, $P=0.03$). In the case
 161 of bacteraemia, ferritin $\geq 1,500$ µg/l (RR 6.1, CI 95% 1.7–
 162 22.3, $P=0.006$) and use of TBI (RR 0.09, $P=0.045$) were
 163 independent predictors. Finally, three variables were related
 164 with the number of days with fever: ferritin $\geq 1,500$ µg/l
 165 ($B=3$; CI 95% 1.2 to 4.9; $P=0.002$), autologous vs allo-
 166 transplants ($B=-3.1$; CI 95% -3 to -4.8 , $P=0.001$), and
 167 number of days with $ANC < 500/\mu l$ ($B=0.3$; CI 95% 0.02 to
 168 0.6, $P=0.04$).

169 Pre-SCT ferritin levels were significantly different
 170 between AUTO- (mean ferritin 769 µg/l) and ALLO-
 171 (mean ferritin 1,254 µg/l) transplants ($P=0.03$). For this
 172 reason, we tried to analyse the impact of pre-SCT ferritin
 173 levels in early infection independently, according to the
 174 transplant type (AUTO vs ALLO). In the group of AUTO-
 175 transplants, ferritin $\geq 1,500$ µg/l was a univariate predictor
 176 for mucositis (50 vs 17%, $P=0.05$), bacteraemia (50% vs
 177 17%, $P=0.05$) and number of febrile days (9 vs 4,
 178 $P<0.001$). Ferritin $\geq 1,500$ µg/l retained its predictive value
 179 in multivariate analysis in this group of patients for severe
 180 mucositis (RR 2.3, $P=0.028$, CI 95% 1.3 to 79), bacter-
 181 aemia (RR 1.6, $P=0.05$, CI 95% 1.003 to 25) and days with
 182 fever ($B=5$, $P<0.001$ CI 95% 2.4 to 7.6). In the group of
 183 ALLO transplants, patients with levels of ferritin above
 184 1,500 µg/l had a slightly higher frequency of severe
 185 mucositis, bacteraemia and days with fever, but these
 186 differences were not significant in univariate or in multi-
 187 variant analysis.

188 Because there were very few C282Y homozygous (0)
 189 and C282Y/H63D compound heterozygous (2) patients in
 190 our sample, we were unable to study whether there was any
 191 relationship between this genetic variable and toxic-infec-
 192 tious complications.

Discussion

193 Mucositis is common after SCT and can be partly mediated
 194 by non-transferrin-bound iron (NTBI) during cytotoxic
 195 chemoradiotherapy, as has been observed in children
 196 undergoing chemotherapy for ALL [7]. Moreover, the
 197

198 mechanisms involved in natural resistance to infection can
 199 only function successfully in an environment where the
 200 normal concentration of free ionic iron is about 10^{-18} M [5],
 201 which can be regarded as virtually zero. For this reason,
 202 hypoferraemia is a normal response to infection and appears
 203 to be part of a natural resistance mechanism [9]. As shown in
 204 experimental infection, bacterial virulence may increase
 205 when there is additional free iron in the medium [10]. In
 206 this context, increased TS and ferritin may be risk factors for
 207 the development of systemic bacterial and fungal infections
 208 in patients with haematological malignancies [12]. An
 209 increase in some late fungal infections, especially mucormy-
 210 cosis, has been reported in iron loaded patients after SCT
 211 [13]. Several articles have suggested that IO can increase
 212 infectious deaths in SCT [1, 2, 15].

213 In the present study, we found a clear relationship
 214 between pre-SCT ferritin levels and the three early toxic-
 215 infectious complications studied. Moreover, a “dose-effect”
 216 relationship was observed between the ferritin level and the
 217 appearance of severe mucositis and bacteraemia. Neverthe-
 218 less, this relationship was fundamentally restricted to
 219 AUTO-transplanted patients. One explanation for this
 220 finding is that other important variables may influence the
 221 appearance of infection in ALLO-transplanted patients such
 222 as, for example, the graft-versus-host disease (GVHD)
 223 prophylaxis used or the appearance of GVHD and its
 224 treatment. We recently described a relationship between
 225 invasive *aspergillus* infection and body iron levels in
 226 transplanted patients [2], but this relationship was also
 227 especially intense in AUTO-transplant patients. Interest-
 228 ingly, Miceli et al. [15] found a similar effect in a larger group
 229 of AUTO-transplanted patients.

230 Ferritin is an acute phase reactant and is not the method
 231 of reference to measure iron load, but a liver biopsy is
 232 difficult to perform in transplant patients, and non-invasive
 233 and accurate MRI-based methods to measure liver iron are
 234 not extensively available. In our opinion, the fact that all
 235 patients in this study were in CR-OR and without
 236 chemotherapy treatment a minimum of 3 months before
 237 SCT suggests that levels of ferritin were a good estimator of
 238 body iron levels and were not significantly interfered by an
 239 “inflammatory state”.

240 We did not find any relationship between TS and the three
 241 toxic-infectious complications studied. This result is surpris-
 242 ing because it is generally accepted that NTBI is the biological
 243 form of iron directly related with promotion of toxicity and
 244 infection, and it has previously been demonstrated that it is
 245 precisely in patients with a $TS \geq 80\%$ in whom NTBI usually
 246 appears [16]. TS levels during conditioning and after
 247 transplant may perhaps be non-equivalent parameters, the
 248 first acting as a surrogate marker of early toxicity of the
 249 conditioning and the second directly related with the period
 250 of erythropoietic arrest after SCT [4].

251 These results support the hypothesis that iron burden can
 252 act as a chemical catalyser of free radicals, causing
 253 increased toxicity (mucositis) and promoting early infec-
 254 tious events in AUTO-transplanted patients. If these results
 255 are reproduced in future studies, pre-transplant manoeuvres
 256 to decrease IO [8] should be proposed to decrease adverse
 257 events and improve survival.

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