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### Serum hepcidin following autologous hematopoietic cell transplantation: an illustration of the interplay of iron status, erythropoiesis and inflammation

Hepcidin, the key hormone in iron homeostasis,<sup>1</sup> is regulated by erythropoietic activity and hypoxia (negative feedback),<sup>2</sup> as well as iron stores (hepcidin induction by iron) and inflammation (upregulation).<sup>1,3</sup> Only a few publications have focused on hepcidin in the context of hematopoietic cell transplantation (HCT).<sup>4,7</sup> We hypothesized that changes in erythropoietic activity (myelosuppression followed by engraftment) and iron parameters (raised ferritin and transferrin saturation (TSAT)) following conditioning and transplantation had an impact on hepcidin levels.

One hundred and twenty-seven patients were included in our randomized trial comparing no erythropoietic therapy, darbepoetin alpha (DA) therapy (300 µg QOW from Day 28 to Day 112 posttransplant) in the DA group and DA (same DA schedule) + intravenous (iv) iron sucrose (200 mg on Days 28, 42 and 56) in the DA + iron group, following autologous HCT.<sup>8</sup> From among these, we randomly selected 15 patients in each group. Patients' characteristics are summarized in Table 1. Details about transfusion thresholds, blood analyses and patient eligibility are reported elsewhere.<sup>8</sup> Erythropoietic activity was evaluated by soluble transferrin receptor (sTfR). Serum hepcidin-25 was quantified on serum samples stored before conditioning and on Days 7, 14, 28, 60, 100 and 180 post transplant, by weak cation exchange time-of-flight mass spectrometry.<sup>9</sup>

Figure 1A shows serum hepcidin over time in the 3 groups. Before HCT, hepcidin values were 2.53±3.16

(mean ± standard deviation), 3.93±2.99 and 5.02±4.35 nmol/L in the control, DA and DA + iron groups, respectively (NS). Hepcidin peaked seven days after HCT, followed by a rapid decrease until Day 28. Thereafter, hepcidin levels in the control group remained stable, whereas those in DA groups decreased rapidly until Day 60. This decrease was better illustrated with hepcidin expressed as a percentage of Day 28 value (before DA treatment) (Figure 1B). From Day 60, hepcidin levels in the DA + iron group increased again and surpassed those in the control and DA groups on Day 100. Two-way analyses of variance (ANOVA) confirmed the effect of treatment group (control vs. DA vs. DA + iron groups) and posttransplant time on hepcidin levels ( $P<0.001$ ). Hepcidin levels prior to transplantation and on Days 14 and 28 correlated with nearly all subsequent ones except with Day-7 values ( $P<0.01$ ).

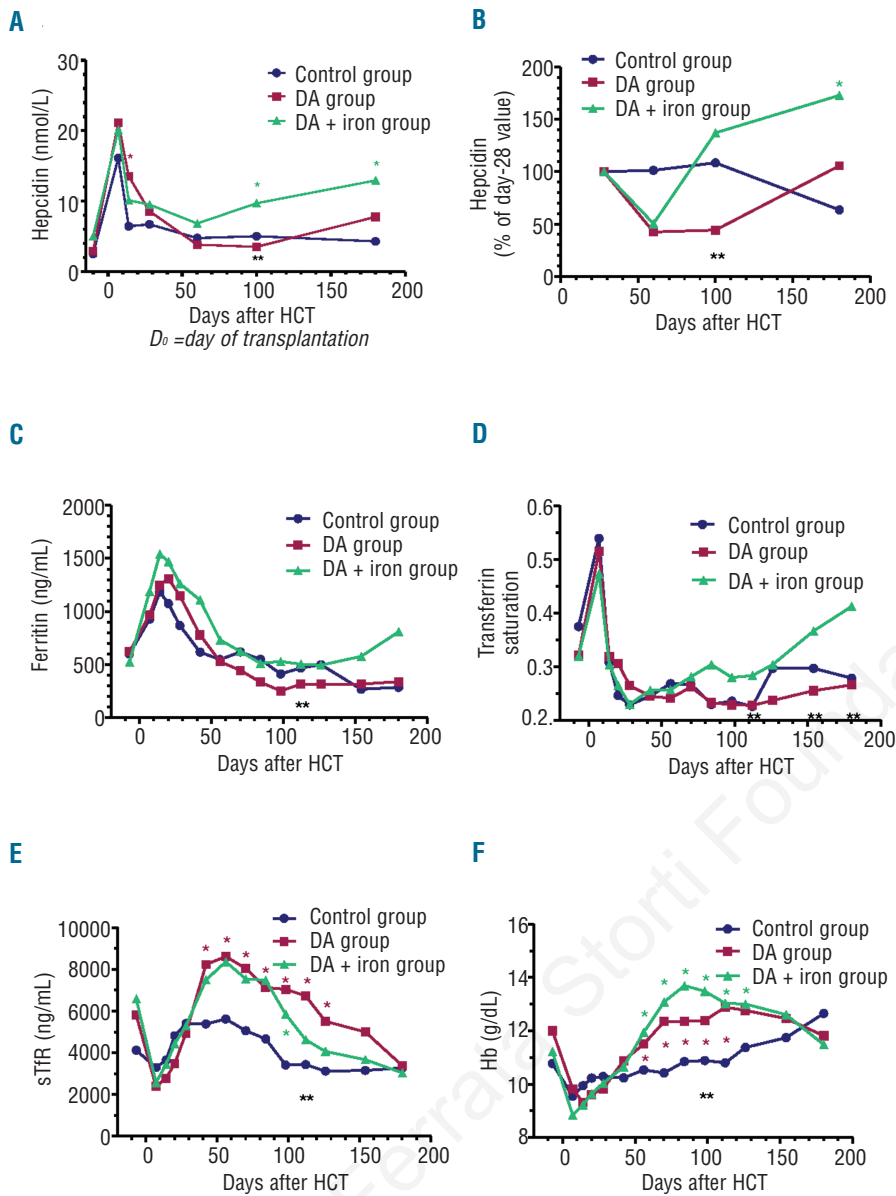
Figures 1C, 1D, 1E and 1F show the evolution over time of ferritin, TSAT, sTfR and Hb, respectively. Two weeks after HCT, ferritin peaked in the 3 groups, then decreased. TSAT was highest on Day 7, then decreased until Day 28. After DA initiation on Day 28, TSAT tended to increase in the DA + iron group. sTfR levels dropped on Day 7 due to conditioning, followed by a sharp increase with engraftment. After DA initiation, sTfR increased in both DA groups, but levelled off in the control group.

All hepcidin values except those on Day 7 correlated with preceding, same day or subsequent ferritin levels; correlations were strongest when examined in same day samples, as well as on Days 60 and 100 ( $r$  approx. 0.75,  $P<0.0001$ ). Otherwise, hepcidin weakly correlated with TSAT ( $r$  between 0.32 and 0.65,  $P$  values ranging from 0.05 to  $<0.0001$ ) and negatively with sTfR (on Days 60 and 100:  $r$  between -0.41 and -0.58,  $P<0.02$ ) and reticulocytes (only on Day 100,  $r=-0.42$ ,  $P=0.004$ ). Finally, no significant correlations were observed between hepcidin and C-reactive protein.

Table 1. Patients' characteristics.

	Control group	DA group	DA + iron group	Reference values
N. of patients	15	15	15	
Age (mean ± SD)	54±10	58±5	54±11	
Sex (F/M; #)	7/8	3/12	4/11	
Diagnosis (#)				
Multiple myeloma	9	8	7	
Hodgkin lymphoma	2	0	2	
High-grade NHL	3	5	4	
Low-grade NHL	1	2	2	
Status at transplantation (#)				
1 <sup>st</sup> or 2 <sup>nd</sup> complete remission	6	7	8	
1 <sup>st</sup> or 2 <sup>nd</sup> partial remission	5	4	6	
Beyond 2 <sup>nd</sup> partial remission	3	3	0	
Undetermined	1	1	1	
Conditioning regimen (#)				
Melphalan 200 mg/m <sup>2</sup>	9	8	7	
BEAM	4	3	4	
Cy-TBI	2	3	4	
Other	0	1	0	
Pre-transplant values (mean ± SD)				
Hemoglobin	10.8±2.4	12.0±1.9	11.2±1.6	12-17 g/dL
Ferritin	601±563	620±639	525±478	25-300 ng/mL*
Transferrin saturation	0.38±0.08	0.32±0.06	0.32±0.06	0.20-0.45
sTfR	4,122±1,130	5,808±2,772	6,577±2,770	3,000-7,000 ng/mL
Serum hepcidin	2.53±3.16	2.93±2.99	5.02±4.35	<0.5-15.6 nmol/L*

DA: darbepoetin alpha; SD: standard deviation; NHL: non-Hodgkin lymphoma; BEAM: BCNU, etoposide, cytarabine, melphalan; Cy: cyclophosphamide; TBI: total body irradiation. \*Depending on gender and pre- and post-menopausal status.



**Figure 1.** Temporal evolution of mean hepcidin (absolute values (A) and percentage of Day-28 values (B)), ferritin (C), transferrin saturation (D), sTfR (E) and hemoglobin (F) following transplantation in the 3 groups. \*Comparisons between either the DA group (\*in red) or the DA + iron group (\*in green) and the control group,  $P < 0.05$ . \*\*Comparisons between the DA group and the DA + iron group,  $P < 0.05$ .

tive protein (CRP), Hb, Hct, percentages of hypochromic red cells and erythropoietin. In multiple linear regression, we found that ferritin was the independent variable most commonly associated with hepcidin from Day 14 to 180 (Table 2). Erythropoiesis (reticulocytes) was also an important determinant of hepcidin on Days 7 and 100. Interestingly, CRP had also an impact on hepcidin on Days 60 and 100.

Hepcidin is the key regulator of iron metabolism but has been little investigated in the context of HCT.<sup>4,7</sup> Hepcidin peaked at Day 7 post transplant and then gradually decreased in the following three weeks without returning to pre-transplant levels.<sup>5</sup> In another study,<sup>6</sup> hepcidin levels at 3-5 months post transplant remained unchanged compared to (high) pre-transplant values. On the other hand, hepcidin response to erythropoietin therapy has been examined in healthy volunteers<sup>10</sup> or chronic kidney disease<sup>10,11</sup> in whom erythropoietin (EPO) elicited a rapid and persistent drop in serum hepcidin levels. Therefore, we decided to investigate the long-term kinetics of hepcidin

after HCT in a group of patients participating in a clinical trial of post-transplant DA with and without intravenous iron therapy.<sup>9</sup> In our study, hepcidin levels prior to HCT were not increased compared to healthy controls.<sup>9</sup> This is in agreement with a previous study<sup>7</sup> and is explained by the low transfusion rates in our lymphoma and myeloma patients before transplantation, contrarily to other studies investigating mostly polytransfused patients with acute leukemia or myelodysplastic syndromes.<sup>4-6</sup> We also identified a hepcidin peak one week after transplantation. Whereas Kanda attributed this peak to inflammation because of a concomitant elevation of serum IL-6 levels (not measured in our study),<sup>5</sup> and although CRP in our study also peaked at Day 7 (*data not shown*), we did not observe any correlation between hepcidin and CRP values.

Among factors associated with hepcidin regulation, erythropoietic activity and iron status are essential.<sup>1,3,12</sup> We also identified erythropoiesis and iron as major determinants of serum hepcidin levels. The Day-7 peak (Figure 1A) could result from concomitant suppression of erythropoietic

**Table 2.** Multiple linear regression of various parameters with hepcidin as the dependent variable at each time point after HCT (group, Hb, sTfR, reticulocytes, ferritin, transferrin saturation (TSAT) and CRP were included into the model).

	Correlation
Before conditioning	None
Day 7	Reticulocytes ( $P=0.021$ )
Day 14	Reticulocytes ( $P=0.008$ )
	Ferritin ( $P=0.029$ )
Day 28	Ferritin ( $P<0.001$ )
Day 60	Ferritin ( $P<0.001$ )
	CRP ( $P=0.003$ )
Day 100	Ferritin ( $P<0.001$ )
	Reticulocytes ( $P=0.002$ )
	CRP ( $P<0.001$ )
	Group ( $P<0.01$ )
	TSAT ( $P=0.011$ )
Day 180	Ferritin ( $P=0.023$ )

activity and elevated serum ferritin levels (due mainly to the drop in iron utilization from the bone marrow and inflammatory complications) (Figure 1C). However, we cannot exclude the possibility that ferritin increased because of iron retention in hepatocytes and macrophages following the hepcidin peak. The ensuing rapid hepcidin drop appears to correspond to fast recovery of erythropoiesis, as illustrated by the quick TSAT normalization (Figure 1D) and sTfR surge (Figure 1E), whereas serum ferritin decreased much slower. This rapid hepcidin decline is coherent with the week-long hepcidin suppression following a single EPO injection observed by Ashby<sup>10</sup> in healthy volunteers. In addition, analyzing hepcidin relatively to its Day-28 (pre-DA) value (Figure 1B) clearly showed that patients receiving no DA had stable values while patients receiving DA experienced a marked drop corresponding to further stimulation of erythropoiesis, whereas TSAT and ferritin evolution remained superimposable in the 3 groups. Obviously, our observations also support the experimental evidence that suppression of hepcidin during anemia requires erythropoietic activity.<sup>13</sup>

Hepcidin values showed significant correlations with all preceding or subsequent values, suggesting that whatever the changes in erythropoietic activity, hepcidin remained under the influence of a less variable parameter, i.e. iron stores. The most robust associations were found between serum hepcidin and ferritin, whereas those with sTfR were less consistent, but the correlations were stronger prior to and away from the day of transplantation, when inflammatory complications had resolved and erythropoiesis recovered. Moreover, in the DA + iron group, hepcidin increased from Day 60 onwards (Figure 1B), in parallel to the evolution of ferritin and TSAT.

In summary, the evolution of serum hepcidin prior to and following autologous HCT in our study was determined by the levels of both erythropoietic activity and iron stores, while the effect of inflammation was less apparent.

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doi:10.3324/haematol.2013.100032

Key words: autologous hematopoietic cell transplantation, darbepoetin alfa, erythropoietin therapy, iron, hepcidin.

Acknowledgments: FB is senior research associate of the National Fund for Scientific Research (FNRS) and AJ is (FNRS)-Televie PhD student. We are grateful to Yvette Fairon and Olivier Dengis for their excellent technical assistance.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at [www.haematologica.org](http://www.haematologica.org).

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