

Erythropoiesis and iron metabolism after haematopoietic stem cell transplantation

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SUMMARY

After haematopoietic stem cell transplantation (HCT), many patients present anaemia, which can persist for months due to an inadequate erythropoietin production for the degree of the anaemia. In this thesis, we performed two randomised studies with erythropoiesis-stimulating agents therapy after allogeneic (including myeloablative and non-myeloablative conditioning) and autologous transplantation. We showed a great efficacy of this growth factor to ensure full erythroid reconstitution when initiated soon after engraftment and not immediately after the transplant. Furthermore, as iron parameters are quite disturbed following HCT, we sought to study iron metabolism after HCT (which has not been much investigated), integrating the role of hepcidin, the key regulator in iron metabolism. Hence, we demonstrated that hepcidin levels prior to and following autologous HCT were influenced by iron stores and changes in erythropoietic activity.

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INTRODUCTION

Erythropoietin (Epo), mainly produced by the kidney, is the major growth factor that stimulates erythropoiesis, even though vitamins, thyroid hormones, androgens and other growth factors like stem cell factor, Flt-3 ligand, thrombopoietin, interleukin (IL)-11, IL-3 and granulocyte-monocyte colony-stimulating factor are also involved.¹ Epo expands erythropoiesis mainly by preventing apoptosis of erythroid progenitors and proerythroblasts.² Epo is therefore unlikely to increase red blood cell (RBC) production when endogenous Epo is elevated and progenitors are already surviving and differentiating.

Haematopoietic stem cell transplantation (HCT) is a treatment able to cure haematological malignancies such as leukaemia, lymphoma and some non-malignant haematological disorders.³ Following HCT, serum Epo levels go through successive phases: there is an initial surge right after the conditioning (due to a lack of utilisation by erythroid precursors),⁴ after which Epo levels decrease to normal range after marrow recovery.⁵ After allogeneic HCT (allo-HCT),

this is followed by a prolonged Epo defect in the post-engraftment period. This inadequate Epo production for the degree of anaemia from day 28 was initially observed in allogeneic but not in autologous bone marrow transplantation.⁶ However, as peripheral blood stem cell (PBSC) became the most frequently used graft source, it was also observed that serum Epo levels may also be transiently inadequate following autologous PBSC transplantation.⁵ Finally, contrarily to myeloablative (MA) allo-HCT, serum Epo levels remain adequate throughout the post-transplant course after non-myeloablative (NMA) conditioning.⁷ Therefore, treating post-HCT anaemia with erythropoiesis-stimulating agents (ESA; of which recombinant human Epo [rhEPO]) could be successful.

On the other hand, iron metabolism is quite disturbed following HCT. Indeed, ferritin levels were found to be considerably increased following HCT because of inflammatory complications and iron shifting into stores.⁸ Likewise, after conditioning, transferrin saturation is often high and decreases only with recovery of erythropoietic activity.⁹

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Recently, hepcidin was found to be the key hormone in iron homeostasis.¹⁰ Heparin is regulated by erythropoietic activity and hypoxia (negative feedback), as well as iron stores (hepcidin induction by iron) and inflammation (upregulation).¹⁰⁻¹² However, since its discovery, only a few publications focused on hepcidin in the context of HCT.¹³⁻¹⁵

ERYTHROPOIESIS AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION

Many randomised trials have failed to show a clear benefit of rhEPO treatment after allo-HCT and even no benefit at all following auto-HCT.¹⁶⁻²¹ However, the pathophysiology of erythropoiesis was not considered in these trials on rhEPO therapy following allo-HCT. Indeed, rhEPO was started on day one, maintained during four to eight weeks or until erythroid engraftment and administered intravenously at very high doses (usually greater than 1000 U/kg/week), leading to a prohibitive cost. In these randomised studies, rhEPO promoted erythropoiesis engraftment but reduced RBC transfusion needs only inconstantly, for example, only between days 20 and 40 (but not overall) and particularly in case of severe graft-versus-host disease in the largest trial. Therefore, soaking patients with very high doses of rhEPO, when the erythroid marrow has not developed enough erythroid precursors and endogenous Epo levels are appropriate or excessive for the degree of anaemia, may not be ideal.

Consequently, our team took a more physiological approach and showed in a pilot study that rhEPO could be very efficient when rhEPO therapy was started 35 days after MA allo-HCT at 500 U/kg/week, with the haemoglobin (Hb) response rate exceeding 90%.²² Moreover, another pilot trial conducted after NMA transplantation demonstrated that rhEPO therapy was also efficient after NMA transplantation, but less than after conventional HCT.²³

This prompted us to conduct two randomised trials to assess the Hb response and transfusion requirements after allogeneic and autologous transplantation with or without ESA.^{24,25}

ERYTHROPOIETIN THERAPY AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION: A PROSPECTIVE RANDOMISED TRIAL

One hundred and thirty-one patients undergoing allogeneic transplantation were randomised in two arms: the control arm (no treatment) and the rhEPO arm (erythropoietin β at the dose of 500 U/kg/week).²⁴ Patients were stratified for the type of conditioning (MA vs NMA) and for the start (day 0 or day 28) of rhEPO therapy for NMA transplantation. Sixty-three per cent of patients receiving rhEPO, versus 8% of con-

trols, achieved the primary endpoint (complete Hb correction, namely Hb \geq 13 g/dl) at a median of 90 days (*Figures 1A and 1B*). Hb levels were higher in the rhEPO arm from week two after treatment initiation, but the difference was significant only from day 60 (*Figure 1C*). This was also true in sub-analyses of each cohort. RBC transfusion requirements were also reduced (both proportions of transfused patients and number of RBC units transfused by patient; *Figure 1D*), even though, analysed separately, this was only significant in the NMA cohort receiving rhEPO from day 28. This confirms the findings of our pilot trials after MA conditioning and following NMA transplantation.^{22,23} We also asked whether there was any benefit to start rhEPO earlier (day 0 rather than day 28) after NMA transplantation. There was no significant difference between the two rhEPO groups during days 0-30, days 30-126 or overall, neither for proportions of patients transfused nor for numbers of units transfused per patient. Unfortunately, no conclusion could be drawn on the effects of rhEPO treatment on anaemia-related symptoms, because many patients failed to return their quality of life (QoL) questionnaires.

The most important limitation of this study was the target Hb value of 13 g/dl. Indeed, current guidelines recommend a target Hb around 12 g/dl because of the possible association between high Hb levels and adverse outcomes.²⁶ Our aim was to normalise Hb to rapidly achieve QoL improvement and transfusion independence. Nevertheless, even if we had adopted a much lower target Hb such as 9 g/dl, this would not have modified the impact of rhEPO on transfusions, because their trigger was 8 g/dl.

Despite this limitation, our study is the first prospective randomised trial to demonstrate rhEPO efficacy in terms of Hb responses and limitation of transfusions in allogeneic transplantation after MA or NMA HCT. However, there was no benefit in initiating treatment earlier, even in the case of NMA conditioning, thereby confirming – in the setting of NMA transplantation – previous results obtained after MA transplantation.

DARBEPOETIN-ALPHA AND INTRAVENOUS IRON ADMINISTRATION AFTER AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION: A PROSPECTIVE MULTICENTRE RANDOMISED TRIAL

Following the same rationale as in the allogeneic setting, our group conducted a phase II trial and a small randomised trial with promising results when treatment is begun soon after engraftment (around day 30 after autologous HCT [auto-HCT]).^{27,28} Furthermore, in our pilot study, several patients had a functional iron deficiency that can efficiently

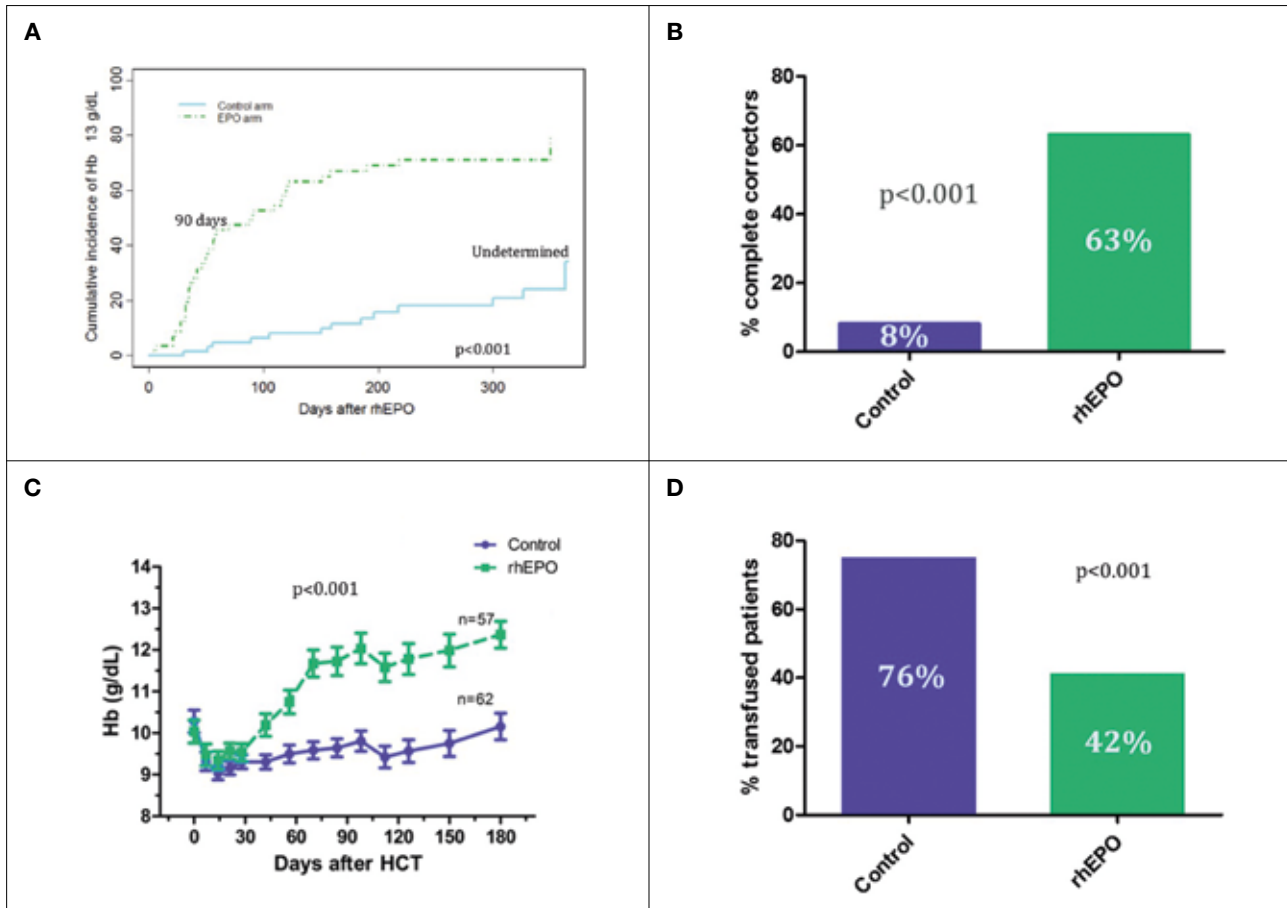


FIGURE 1. Randomised trial of recombinant human erythropoietin therapy after allogeneic haematopoietic stem cell transplantation. Cumulative incidence of patients reaching haemoglobin ≥ 13 g/dl (**A**), proportions of patients correcting their haemoglobin (**B**), haemoglobin levels following haematopoietic stem cell transplantation (**C**) and proportions of patients transfused during the study (**D**) in the two arms ($n=62$ in control arm and $n=57$ in recombinant human erythropoietin arm).

Hb: haemoglobin, EPO: erythropoietin, rhEPO: recombinant human erythropoietin, HCT: haematopoietic stem cell transplantation.

be treated with intravenous (IV) iron. This led us to conduct a multicentre prospective randomised study analysing the impact of darbepoetin-alpha (DA) administration with or without IV iron on erythroid recovery after auto-HCT.²⁵

The study was a three-arm multicentre randomised study of DA administration after auto-HCT. Subjects in group 1 received neither DA nor IV iron; those in group 2 received DA alone starting on day 28 after the transplant; while those in group 3 received DA and IV iron saccharate, both starting at day 28 after the transplant (25 subjects in each arm with randomisation 1:1:1), then after an amendment, 25 subjects were added to group 2 and 3 to increase the statistical power of the study.

We showed that DA was remarkably efficient to promote erythropoiesis with 79% of patients receiving DA alone versus 21% of controls achieving the primary endpoint of the study (complete Hb response, namely ≥ 13 g/dl; Figure

2A), at a median of 40 and 190 days, respectively. The proportions of patients requiring RBC transfusions or the mean numbers of transfusions received between day 28 and day 126 after HCT were very low and not different between the three groups (Figure 2B). Finally, Hb levels were significantly higher in the two DA groups compared to group 1 from day 42 through day 150 after the transplant (Figure 2C).

In the comparison between the two DA groups (with or without IV iron), we observed a higher proportion of patients reaching Hb levels ≥ 13 g/dl before day 126 (82% in group 2 and 98% in group 3; Figure 2D), at a median of 45 and 31 days, respectively ($p=0.008$). More patients in group 2 required RBC transfusions ($p=0.03$), but the mean numbers of transfusions received between day 28 and day 126 after HCT were not different between the two groups (Figure 2E). At last, patients had higher Hb values in group 3 than in group 2, from day 70 to day 112 after HCT (Figure 2F).

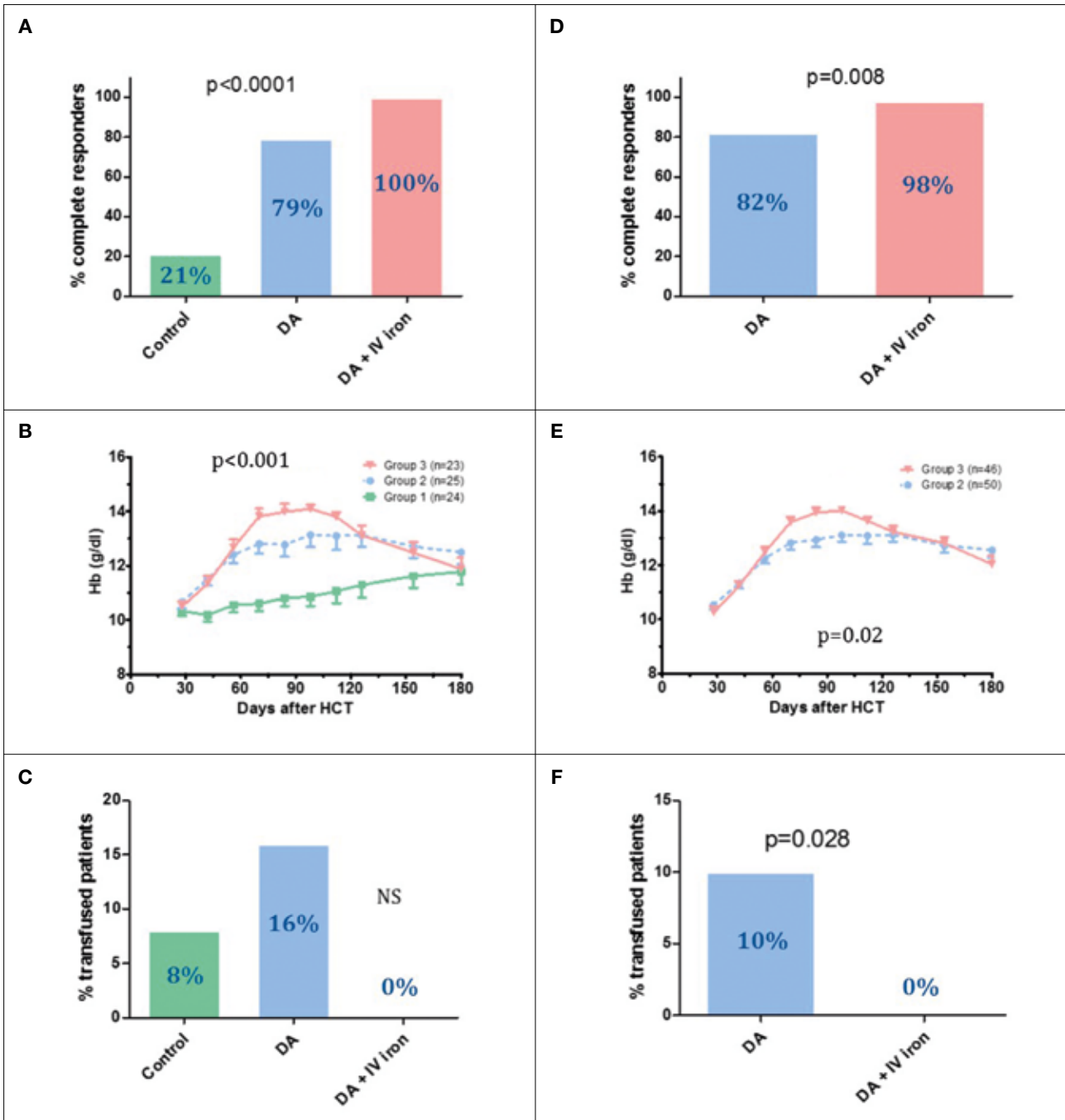


FIGURE 2. Randomised trial of erythropoiesis-stimulating agents therapy ± intravenous iron after autologous haematopoietic stem cell transplantation. Proportions of patients correcting their haemoglobin (A), haemoglobin levels following haematopoietic stem cell transplantation (B) and proportions of patients transfused during the study (C) in the three groups (n=25 each). Proportions of patients correcting their haemoglobin (D), haemoglobin levels following haematopoietic stem cell transplantation (E) and proportions of patients transfused during the study (F) in the two darbepoetin-alpha groups (50 patients in each group). Group 1= control group, group 2= DA group and group 3= DA + iron group. DA: darbepoetin-alpha, IV: intravenous, Hb: haemoglobin, HCT: haematopoietic stem cell transplantation.

We also compared the total dose of DA given in the two DA groups, and it was lower in group 3 ($1.210 \pm 401 \mu\text{g}$) than in group 2 ($1.440 \pm 496 \mu\text{g}$) ($p=0.015$). As in the allogeneic trial, we can discuss about the target

Hb value of 13 g/dl that was higher than current recommendations on ESA administration.^{26,29} However, such a limitation on the target Hb was not effective at the time of the trial in 2004.

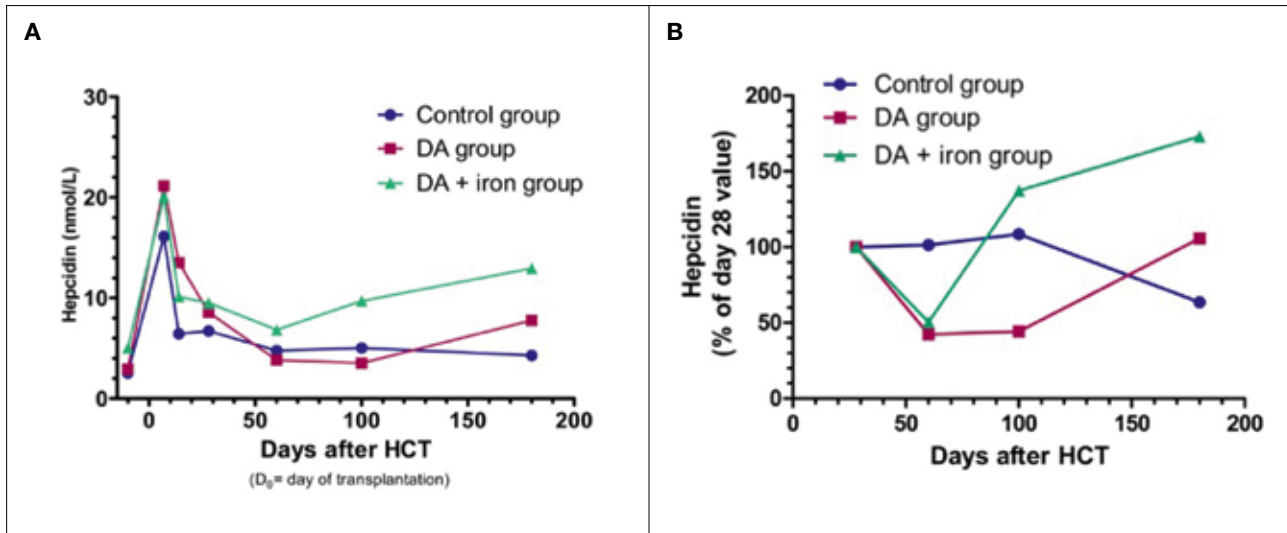


FIGURE 3. Temporal evolution of mean hepcidin levels following autologous haematopoietic stem cell transplantation in the three groups (absolute values **[A]** and percentages of day-28 values **[B]**).

DA: darbepoetin-alpha, HCT: haematopoietic stem cell transplantation.

Despite this limitation, our data indicate that DA, when started on day 28 post-transplant, is safe and highly effective to ensure full erythroid reconstitution after auto-HCT but should probably be used only if the transfusion risk is significant.

LONG-TERM SAFETY ANALYSES

Following some safety concern of ESA (highlighted in the anaemia of cancer), it is now essential to rule out a prospective effect of ESA on relapse and survival.^{30,31} Therefore, we performed long-term analyses after our two randomised trials to verify the safety of ESA after HCT.^{32,33} Importantly, we demonstrated that rhEPO therapy after allo- and auto-HCT was safe in short- and long-term (more than eight years in survivors) analyses since survival was the same in ESA and control arms.^{32,33} Specifically, the relapse incidence and the rate of thromboembolic and cardio-vascular events were not increased in ESA arms. However, in contrast to other studies on ESA in cancer patients with active malignancies, the major part of our population was in complete remission before HCT or achieved it quickly. Furthermore, no negative influence of IV iron on tumour progression or infection rates was found.

IRON METABOLISM AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION

In the last part of our work, we analysed the evolution of hepcidin in relation with erythropoietic activity, inflammation and iron parameters, the usual hepcidin regulators, following

auto-HCT in a subgroup of patients included in the second study (15 patients in each group of the trial about ESA \pm IV iron after auto-HCT).³⁴ We found a hepcidin peak on day 7 post-HCT, followed by a progressive decline in the next three weeks (Figure 3A). 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 the day-28 value (before DA treatment, Figure 3B). While previous studies attributed the day-7 peak to inflammation due to an increase in IL-6 levels at the same time, no correlation was found between hepcidin and inflammation (assessed by C-reactive protein levels), except in multiple linear regression limited on days 60 and 100 post-HCT.¹³ Hepcidin was rather associated with erythropoietic activity as the suppression of erythropoiesis occurring after the conditioning led to increased hepcidin values and hepcidin levels were sensitive to the stimulation of erythropoiesis in patients receiving ESA. Furthermore, iron stores were also a major determinant for hepcidin regulation since strong correlations were observed between hepcidin and ferritin levels, particularly away from the day of HCT.

CONCLUSION

ESA therapy is efficient and safe after allo- or auto-HCT, when initiated from day 28 post-transplant. In autologous setting, IV iron administration allowed an acceleration of erythroid recovery and enhanced the response rate to ESA, with a possible improvement of QoL. Larger trials with better assessment of QoL are needed. In addition, analyses of iron

KEY MESSAGES FOR CLINICAL PRACTICE

- 1 After allogeneic haematopoietic stem cell transplantation (HCT), erythropoiesis-stimulating agents (ESA) from day 28 post-transplant are efficient to enhance erythroid reconstitution and to reduce transfusion requirements, whatever the intensity of conditioning. However, there is no benefit to start ESA sooner after non-myeloablative transplantation.**
- 2 After autologous HCT, ESA therapy is highly effective to ensure full erythroid reconstitution but does not reduce transfusion needs. Furthermore, the addition of intravenous iron allows further acceleration of erythroid recovery, improves response rates, reduces ESA requirements and improves quality of life.**
- 3 ESA use after allogeneic or autologous HCT is safe. Intravenous iron in autologous HCT does not induce any additional toxicity (particularly infections and death) in this setting.**
- 4 Hepcidin levels prior to and following autologous HCT are mainly determined by iron stores and erythropoietic activity; inflammation has much less impact on them.**

metabolism following auto-HCT demonstrated that iron stores and changes in erythropoietic activity (due to conditioning then engraftment) influenced hepcidin levels, with a minor effect of inflammation.

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