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Iron overload in hematologic malignancies and outcome of allogeneic hematopoietic stem cell transplantation

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Iron overload is associated with organ toxicity and increased susceptibility to infection. Transfusional iron overload is a frequent complication of the therapy of hematologic malignancies, best exemplified in myelodysplastic syndrome (MDS). The risks of iron overload may be further increased in the context of hematopoietic stem cell transplantation (HSCT). Retrospective studies of patients who have undergone allogeneic HSCT have documented that pre-transplant red blood cell (RBC) transfusion-dependence and/or elevated serum ferritin levels (surrogate measures of iron overload) are associated with poorer post-transplant survival among patients with MDS and acute leukemias. A report by Alessandrino *et al.*,[1] published in this issue of this journal, adds to the debate by more precisely quantifying the impact of transfusional iron overload on allogeneic HSCT outcomes. In this perspective article, we review the literature related to iron overload, hematologic malignancies and allogeneic HSCT and suggest issues for future research.

Iron overload and hematologic malignancies

In benign hematologic disorders (e.g., thalassemia) iron overload due to increased iron absorption and hepcidin suppression, possibly from elevated growth differentiation factor 15 (GDF15) levels,[2] is common and results in toxicity and impaired survival. The toxicity of iron overload is related in part to intracellular generation of free radicals, resulting in oxidative damage and organ dysfunction (e.g. hepatotoxicity, cardiotoxicity, endocrine dysfunction), and in part to increased susceptibility to infection resulting from suppression of host immune responses, and from iron's role as an essential cofactor for pathogen growth.[3,4]

In hematologic malignancies, iron overload is a concern in those disorders associated with a relatively prolonged clinical course, ineffective erythropoiesis, increased iron absorption (possibly from elevated GDF15), and/or the need for multiple RBC transfusions. Although these features are best represented in MDS, in principle any hematologic malignancy associated with multiple RBC transfusions may result in clinically significant iron overload.

While there is some evidence that iron overload is of clinical relevance in acute leukemia,[5] the impact of transfusional iron overload has been best characterized in MDS. Malcovati *et al.* reported that RBC transfusion dependency was an independent negative prognostic factor for overall survival in MDS, and that increasing RBC transfusion load (total transfusions; transfusions per month) was associated with worse survival.[6] RBC transfusion dependency is now incorporated into a WHO-based prognostic staging system (WPSS) for MDS.[7] However, it remains uncertain whether impaired outcomes in MDS patients dependent on RBC transfusions are directly related to iron overload, or to worse MDS biology (e.g., increasing dyserythropoiesis). The association of RBC transfusion dependency with an increased risk of death or progression to acute leukemia suggests that more aggressive MDS biology may be a factor.[6] However, in this analysis, increasing iron overload (defined as a serum ferritin level >1000 ng/mL) was independently associated with worse survival even after discounting the known negative impact of RBC transfusion dependency, suggesting that iron overload may be an independent negative prognostic factor. Interestingly, the adverse impact of elevated ferritin levels was documented only in low-risk subtypes of MDS, such as refractory anemia, with or without ring sideroblasts, rather than in higher risk subtypes such as refractory anemia with multilineage dysplasia or refractory anemia with excess blasts. It is possible that aggressive disease biology trumps iron overload in patients with more advanced MDS. However, given the improved survival of MDS

patients treated with newer disease-modifying therapy (e.g., lenalidomide, hypomethylating agents), the impact of iron-overload on the survival of patients with higher-risk MDS may need to be revisited in the future.

We note that even in MDS, the evidence regarding transfusional iron overload is primarily based on retrospective analyses, and confirmatory prospective trials are necessary. Furthermore, it remains to be determined in prospective, randomized controlled trials whether iron-chelation therapy can safely and effectively reverse iron overload in MDS patients continuing to receive RBC transfusions, and if so, whether this will translate into improved survival.

Iron overload after hematopoietic stem cell transplantation

HSCT recipients are at risk of iron overload from the multiple RBC transfusions received during initial cancer therapy, increased iron absorption related to elevated GDF15 levels, and the peri-transplantation transfusions. Iron overload has been documented after both autologous and allogeneic HSCT.[8-10] While earlier studies reported elevated serum ferritin as an indirect measure of iron overload, more recent studies have documented elevated liver iron concentration (liver biopsy, T2*MRI, R2*MRI) as a direct measure of iron overload after HSCT.[11,12]

Iron overload after HSCT has been associated with both early and late complications. Early complications include infections (invasive fungal infections, such as mucormycosis and aspergillosis, and bacterial infections, for example listeriosis), abnormalities of liver function tests and hepatic sinusoidal obstruction syndrome, which can increase treatment related mortality.[13-19] Late complications of iron overload are ill-defined. While abnormal liver function tests and infections have been reported, hepatic fibrosis/cirrhosis and cardiac dysfunction have not been described except in thalassemic children.[7,12,15]

Iron overload before hematopoietic stem cell transplantation

Armand *et al.* reported a single-institution retrospective analysis of 543 adult patients undergoing myeloablative allogeneic HSCT in whom an elevated pre-transplant serum ferritin level was strongly associated with lower overall and disease-free survival.[20] On subgroup analysis, this association was restricted to patients with acute leukemia or MDS. In the MDS subgroup, inferior survival was attributable to increased transplant-related mortality. Subsequent studies reported variable findings.[21,22] However, a prospective single-institution study of 190 adult patients undergoing myeloablative HSCT documented that elevated pre-transplant serum ferritin was associated with increased hazard of death, 100-day mortality, incidence of acute graft-*versus*-host disease and bloodstream infections or death as a composite endpoint.[23] HSCT toxicity, in the form of hepatic veno-occlusive disease/sinusoidal-obstruction-syndrome, was also significantly increased in patients with elevated serum ferritin levels receiving oral busulfan conditioning.[24]

A multi-institution GITMO retrospective analysis of 365 patients with *de novo* MDS undergoing allogeneic HSCT (245 myeloablative; 120 reduced intensity conditioning) evaluated the impact of pre-transplant WPSS risk score (including pre-transplantation RBC transfusion dependence) on transplantation outcomes.[25] It was found that pre-transplantation dependence on RBC transfusions significantly impaired overall survival after HSCT and was associated with increased transplant-related mortality, especially in patients with lower-risk MDS (without excess blasts). For higher risk MDS (with excess blasts), RBC transfusion dependency had a borderline impact on overall survival and transplant-related mortality. The results were not stratified by type of conditioning (ablative *versus* reduced intensity conditioning).

The GITMO report by Alessandrino *et al.* in this issue of the journal extends those observations in an overlapping population of 357 MDS patients undergoing allogeneic HSCT (217 myeloablative; 141 reduced intensity conditioning).[1] Importantly, it includes data on pre-transplant serum ferritin levels, HSCT comorbidity index, RBC transfusion dependency, and the total number of RBC units transfused. On multivariate analysis, RBC transfusion dependency had an independent, negative effect on overall survival and transplant-related mortality in myeloablative, but not reduced intensity conditioned, HSCT. Of note, this is in contrast to the results of a smaller single-institution retrospective analysis of 64 patients which suggested that elevated pre-transplantation serum ferritin had an independent, negative impact on survival in reduced intensity conditioned HSCT.[26]

An attempt made to determine the impact of increasing iron overload in terms of quantity of RBC transfusions, showed that overall survival and transplant-related mortality were significantly worse in patients undergoing myeloablative HSCT who had received more than 20 packed RBC units pre-transplantation (and further impaired in those who had received more than 40 packed RBC units), while outcomes for patients receiving 20 or fewer packed RBC units were comparable to those of non-transfusion-dependent patients. Additionally, increasing pre-transplant serum ferritin levels were also associated with worse overall survival and transplant-related mortality in the RBC transfusion-dependent patients. In a model combining total RBC transfusion burden, transfusion duration, and serum ferritin level, the independent negative prognostic effect of elevated serum ferritin was maintained, while the number of RBC units transfused did not retain prognostic significance. In part this is consistent with the highly significant correlation shown between increasing pre-transplantation RBC transfusions and elevated serum ferritin level, and suggests that a complete RBC transfusion history is less important than a documented elevated serum ferritin level before transplantation.

This report strengthens the association between transfusional iron overload and impaired survival for MDS patients undergoing myeloablative HSCT. Furthermore, it suggests a threshold iron overload in HSCT, whereby MDS patients receiving more than 20 RBC transfusions have a worse survival. It also documents that elevated serum ferritin levels are strongly correlated with the number of RBC units transfused (i.e., the magnitude of iron loading). Finally, it shows that a single pre-transplantation measurement of serum ferritin level is sufficient for prog-

nostic modeling, and that determining the total number of RBC units transfused may not be necessary.

Prospective confirmation of the association between transfusional iron overload and survival post-HSCT will be important. Also, given the clinical necessity to proceed expeditiously to HSCT in many hematologic malignancies, it will be vital to prospectively determine whether chelation therapy can safely and effectively reduce transfusional iron overload before transplantation, and if this is sufficient to mitigate its deleterious impact. In this regard, an encouraging retrospective report suggests that pre-transplantation iron-chelation to a target serum ferritin level of below 1000 ng/mL can abrogate the negative impact of iron overload in pediatric patients with hematologic malignancies undergoing allogeneic HSCT.[27] Prospectively confirming these findings in adult patients with hematologic malignancies will be an important step in the efforts to improve outcomes after HSCT.

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