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Peritransplantation Red Blood Cell Transfusion Is Associated with Increased Risk of Graft-versus-Host Disease after Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

More than 90% of allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients receive red blood cell (RBC) or platelet transfusions in the peritransplantation period. We tested the hypothesis that transfusions are associated with the development of severe (grade III-IV) acute graft-versus-host disease (aGVHD) or mortality after allo-HSCT in a retrospective study of 322 consecutive patients receiving an allogeneic bone marrow or granulocyte colony-stimulating factor-mobilized blood stem cell graft for a hematologic malignancy. Counting transfused RBC and platelet units between day −7 pretransplantation and day +27 post-transplantation, but excluding transfusions administered after a diagnosis of aGVHD, yielded medians of 5 RBC units and 2 platelet units transfused. Sixty-three patients (20%) developed a maximal grade III-IV aGVHD with onset up to day +150 post-transplantation (median aGVHD onset of 28 days). HLA mismatch (hazard ratio [HR], 2.4; 95% confidence interval [CI], 1.2 to 4.7; \( P = .01 \) ), and transfusion of more than the median number of RBC units (HR, 2.1; 95% CI, 1.1 to 3.7; \( P = .02 \) ) were independently associated with greater risk of grade III-IV aGVHD in a multivariable analysis model. Disease risk strata (HR, 1.7; 95% CI, 1.2 to 2.4 for high risk versus low risk; \( P = .005 \)) and transfusion of more than the median number of RBC units (HR, 1.4; 95% CI, 1.0 to 2.0; \( P = .054 \)) were independently associated with inferior overall survival. These data support our hypothesis that peritransplantation RBC transfusions are associated with the risk of developing severe aGVHD and worse overall survival following allo-HSCT, and suggest that strategies to reduce routine RBC transfusion may favorably reduce the incidence and severity of GVHD.

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INTRODUCTION

Transplantation-related acute graft-versus-host disease (aGVHD) is a major cause of nonrelapse mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and remains a significant barrier to the success of the transplantation procedure [1,2]. Alloreactive donor T cells within the graft initiate an immune attack on recipient target tissues during GVHD pathogenesis [3]. Numerous studies aimed at reducing GVHD severity and improving prognosis following allo-HSCT have reported only modest reductions in the incidence of severe aGVHD and treatment-related mortality [2,4,5]. Successful clinical strategies for GVHD prevention have largely been limited to improved HLA matching and the use of in vivo and ex vivo T cell depletion of the graft [6-9]. However, T cell depletion of the graft has been associated with poor donor immune cell function and higher incidences of disease relapse and opportunistic infections [10].
Risk factors for aGVHD include recipient age, donor sex, conditioning intensity, graft source, HLA match, and donor relation to recipient [11-13]. ABO mismatch between allo-HSCT donor and recipient has been associated with an increased risk of aGVHD in some studied patient populations, but not in others [14,15]. More than 90% of allo-HSCT patients receive red blood cell (RBC) and platelet transfusions just before transplantation and during the first month post-transplantation, but transfusion has not been well studied as a risk factor for adverse outcomes [16-19]. Increased serum ferritin levels due to pretransplantation RBC transfusions were found to be associated with increased risk of GVHD by Pullarkat et al. [20], but this was not found in other studies [21-23]. Preclinical studies in murine allo-HSCT models indicate that RBC transfusions can sensitize transplantation recipients to minor histocompatibility antigens [24-26], whereas clinical studies of platelet transfusion found platelet-specific and anti-human leukocyte antibodies in multiple platelet-transfused patients [27-29]. Thus, third-party transfusions might serve as a source of alloantigen that primes donor T cells, via indirect antigen presentation by donor-derived dendritic cells, to antigens mismatched between the stem cell donor and recipient [25,30], and contribute to an increased risk of aGVHD [31-33].

Here we present the results of a retrospective analysis of the association of RBC and platelet transfusions with GVHD and survival, and show for the first time that larger numbers of RBC transfusions during the peri-transplantation period are associated with increased incidences of maximal grade III-IV aGVHD and inferior overall survival in allo-HSCT recipients.

**MATERIALS AND METHODS**

**Study Endpoints and Selective Criteria**

We conducted an Institutional Review Board-approved retrospective single-center study of 322 consecutive adult patients who received allogeneic bone marrow transplantation (BMT) or granulocyte colony-stimulating factor-mobilized peripheral blood stem cell (PBSC) transplantation from a sibling or unrelated donor between January 2007 and January 2013. A 10/10 allele match was considered a full match, whereas 8/10, or 9/10 allele matches were considered mismatched, following molecular typing at the allele level. All patients had a primary malignant hematologic disease, and patients receiving a haploidentical transplant, a T cell-depleted graft, or an umbilical cord blood transplant were excluded. For patients who received a subsequent allogeneic transplant (n = 9), only transplantation characteristics and transfusion and GVHD data relevant to the first allo-HSCT were included, but survival data and cause of death (if applicable) included status after the second allo-HSCT.

**Karnofsky Performance Status Score, Transplantation, and aGVHD**

Karnofsky Performance Status (KPS) score was determined before admission [34]. Disease risk was classified as low, intermediate, or high according to American Society for Blood and Marrow Transplantation/Center for International Blood and Marrow Transplant Research 2015 standards [35]. Patients were admitted 1 week before allo-HSCT for the conditioning regimen, which was classified as myeloablative (busulfan/ cyclophosphamide or cyclophosphamide/total body irradiation), reduced intensity (fludarabine/melphalan), or nonmyeloablative (fludarabine/total body irradiation). Pretransplantation serum ferritin levels obtained between 5 and 90 days before allo-HSCT (median, 28 days) were available for 299 of the 322 patients.

After transplantation of allogeneic bone marrow or granulocyte colony-stimulating factor-mobilized PBSCs, patients remained hospitalized until hematopoietic engraftment and were monitored in the BMT clinic until day 100. Engraftment was defined according to Center for International Blood and Marrow Transplant Research criteria [36]. Onset of aGVHD within 150 days post-transplantation was graded based on the 1994 Keystone consensus guidelines [37]. For our analyses, patients were assigned to aGVHD groups based on the maximal grade of aGVHD recorded after the initial onset of aGVHD; for example, patients with initial grade II aGVHD that became re-fractory developed grade III or IV aGVHD were analyzed based on the higher assigned grade.

**RBC and Platelet Transfusions**

All RBC and platelet transfusions administered between day –7 and day +27 were provided by the Emory University Hospital Blood Bank after purchase from the American Red Cross Blood Services, Southern Region. RBC units were collected CPD-AS1 or CPDA-1, for a maximum of 42 days or 35 days at 1 to 6 °C, respectively. All RBC and platelet units were also irradiated; RBCs were used within 28 days of irradiation. Standing orders specified transfusion of 2 RBC units when the daily hematocrit value was <27% or 1 irradiated platelet apheresis unit (stored for a maximum of 5 days at room temperature with agitation) when the platelet count was <10 × 10^9/μL. These thresholds remained in effect throughout the study period. Additional platelet units were given at the time of presentation of clinical bleeding, and additional RBC units were given for hemorrhage-induced anemia to maintain a hematocrit of >38%. Pretransplantation erythropoiesis-stimulating agents (eg, darbepoetin, epogen) were not used in these patients.

**Statistical Methods**

Competing-risk endpoints were grade III-IV aGVHD and mortality without the development of grade III-IV aGVHD (patients who died early, up to day +150, without aGVHD or with grade I-II aGVHD, including those diagnosed with chronic GVHD or overlap syndrome) [38]. Of the 25 patients in the early mortality competing-risk group, 13 had no aGVHD, 5 had grade I aGVHD, and 7 had grade II aGVHD. Overlap syndrome chronic GVHD was diagnosed in 2 of the patients in the early mortality group (2 of the 7 patients with grade II aGVHD). We did not exclude patients with de novo chronic GVHD from the early mortality group; however, none of the patients who died up to day +150 with no aGVHD developed de novo chronic GVHD before this time point. The cumulative incidences of grade III-IV aGVHD and early mortality without the development of grade III/IV aGVHD were estimated using the cumulative incidence function [39]. A competing-risk analysis estimated the subdistribution hazard for grade III-IV aGVHD and non-grade III-IV aGVHD mortality using a Cox proportional hazards regression model implemented with SAS PROC PHREG version 9.4 (SAS Institute, Cary, NC). The subdistribution hazard ratio (HR) and its 95% confidence interval (CI) were calculated for each risk factor in univariable analyses. The covariates included in a multivariable analysis was limited to baseline covariates associated with grade III-IV aGVHD at the P < .1 level. Similar Cox regression methods were used for analyses of risk factors potentially associated with all-cause mortality, excluding aGVHD from the multivariable analysis because this is a well-documented cause of death in allo-HSCT recipients. Bootstrap bagging was used to identify stable and reliable predictors of grade III-IV aGVHD. A dataset was constructed of equal size as the original (n = 322) by random sampling of cases with replacement (bootstrap sampling). On average, approximately one-third of subjects were not sampled, whereas some subjects were sampled more than once. The bootstrap sample was analyzed using the Cox model with an automated forward stepwise algorithm with an entry criterion of P < .01 and a removal criterion of P < .05. The result was stored. This process of sampling, automated analysis, and storing was repeated 1000 times. The number of times that a risk factor appeared in these 1000 analyses was taken as reflection of the reliability (signal). Following Brieman’s median rule (devised to balance type I and type II errors), risk factors were determined to be reliably associated with the outcome if they appeared in at least 60% of the models [40,41]. The cause-specific HR and its 95% CI were calculated for each factor in the absence of others in the final model identified with bootstrap bagging. Cumulative long-term survival was estimated with the Kaplan-Meier method. Log-rank tests were used to compare survival by aGVHD grade and by numbers of RBC and platelet units transfused. Causes of death were compared between groups using 2 × 2 contingency tables and Fisher’s exact test.

**RESULTS**

**Patient Characteristics**

A total of 322 consecutive patients who underwent allo-HSCT between January 2007 and January 2013 were studied, excluding patients with nonmalignant underlying disease or recipients of T cell-depleted or umbilical cord blood grafts. The cohort was 55% male and 45% female, with a median age at transplantation of 51 years (range, 19 to 73 years). The most common underlying disease was acute myeloblastic leukemia (41%). Underlying disease risk was low in 41% of patients, intermediate in 26%, and high in 32%. Allografts were obtained from a 10/10 HLA-matched sibling donor in 35% of recipients, from a 10/10 HLA-matched unrelated donor in 43%, and from a donor mismatched at 1 or 2 HLA alleles (3 sibling donors [1%] and 65 unrelated donors [20%]). Graft sources were...
bone marrow in 11% of recipients and mobilized PBSCs in 89%. Recipient and donor characteristics are summarized in Table 1.

**RBC and Platelet Transfusions**

RBC and platelet transfusions were recorded from 1 week before transplantation to 4 weeks after transplantation. Only RBC and platelet units transfused before a diagnosis of aGVHD (any grade) were considered in univariable and multivariable analyses to avoid an indication bias from including units transfused owing to GVHD-related anemia and thrombocytopenia. The median number of RBC units transfused was 5 (range, 0 to 30), with 288 patients (89%) receiving RBC units during this observational time frame and 34 patients (11%) receiving no RBC units. The median duration of storage of RBC units was 19 days (average, 21 days; range, 2 to 42 days). Platelet transfusions were administered to 274 patients (85%), and 48 patients (15%) received no platelet transfusions (median, 2; range, 0 to 34).

**RBC Transfusions Associated with aGVHD**

A total of 239 patients (74%) developed aGVHD with the following maximum grades by day +150 post-transplantation: grade I, n = 106 (33%); grade II, n = 70 (22%); grade III, n = 47 (15%); and grade IV, n = 16 (5%). The calculated cumulative incidence curve for the 63 patients with grade III-IV aGVHD patients is shown in Figure 1A, along with the cumulative incidence of death in the first 150 days without grade III-IV aGVHD as the competing risk (n = 25, including 13 patients with no GVHD, 5 patients with grade I aGVHD, and 7 patients with maximal grade II aGVHD). The median day of onset was day +32 for all grades of aGVHD and day +34 for grade I, day +30 for grade II, and day +28 for grade III-IV aGVHD (Figure 1B). For patients who developed severe grade III-IV GVHD, the median interval between the last RBC transfusion before the diagnosis of aGVHD and the onset of aGVHD was 12 days (range, 1 to 139 days), and that between the onset of GVHD and death was 92 days (range, 6 to 1821 days). The vast majority (90%) of the patients with maximal grade III-IV aGVHD had onset by day +65. Although day +150 was selected as the cutoff time for aGVHD diagnosis to include patients with late-onset aGVHD, only 2 of 63 cases (3%) of grade III-IV acute GVHD had onset between day +100 and day +150 (Supplementary Figure S1).

Univariate analysis identified several risk factors significantly associated with the development of grade III-IV aGVHD (Table 2), including a lower hematocrit value on admission (<25%; P = .02), an HLA-mismatched donor (P = .007), and transfusion of more than the median number of 5 RBC units (P = .001). A longer time to neutrophil engraftment (at or exceeding the median of 15 days) was inversely associated with grade III-IV aGVHD in univariate analyses (Table 2). Of note, higher pretransplantation ferritin level (exceeding the median of 1146 ng/mL) were not associated with an increased risk of developing grade III-IV aGVHD. Multivariate Cox regression analysis including all variables potentially associated with increased grade III-IV aGVHD (P ≤ .2 and HR > 1)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient and Donor Characteristics (n = 322)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Value</td>
</tr>
<tr>
<td><strong>Conditioning regimen, n(%)</strong></td>
<td></td>
</tr>
<tr>
<td>Myeloablative</td>
<td>134 (41)</td>
</tr>
<tr>
<td>Reduced-intensity conditioning</td>
<td>159 (50)</td>
</tr>
<tr>
<td>Nonmyeloablative</td>
<td>29 (9)</td>
</tr>
<tr>
<td><strong>Disease, n(%)</strong></td>
<td></td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>133 (41)</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>46 (14)</td>
</tr>
<tr>
<td>Myeloproliferative syndrome</td>
<td>17 (5)</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>40 (12)</td>
</tr>
<tr>
<td>Acute leukemia, biphenotypic or undifferentiated</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Chronic lymphoid leukemia</td>
<td>15 (5)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>32 (10)</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>7 (2)</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>22 (7)</td>
</tr>
<tr>
<td><strong>Disease risk status, n(%)</strong></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>133 (41)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>85 (26)</td>
</tr>
<tr>
<td>High</td>
<td>104 (32)</td>
</tr>
<tr>
<td><strong>Recipient and/or donor positive, n(%)</strong></td>
<td></td>
</tr>
<tr>
<td>Recipient and/or donor positive</td>
<td>254 (79)</td>
</tr>
<tr>
<td>Both negative/both unknown</td>
<td>39 (12)/29 (9)</td>
</tr>
</tbody>
</table>

KPS indicates Karnofsky Performance Status.
in univariate analysis) revealed that HLA mismatch and more RBC units (but not platelet units) transfused were independently associated with an increased risk of grade III-IV aGVHD (P = .01 and .02, respectively; Table 3). Similar results were obtained when we tested a cutoff time of 100 days post-transplantation rather than 150 days for diagnosis of aGVHD (data not shown).

In univariate analyses for the competing risk of early mortality without grade III-IV aGVHD, several factors were significantly associated with death in this group, including earlier date of transplantation (2007-2009 versus 2010-2012; P = .01), platelet engraftment time equal to or greater than the median of 18 days (P = .005), and transfusion of more than the median number of 2 platelet units (P = .009). We also found that both mobilized PBSC grafts and increased average storage time of transfused RBC units were inversely associated with the competing risk of early mortality (HR, 0.4 [P = .05] and 0.4 [P = .01], respectively; Table 2). Importantly, neither HLA mismatch nor a greater number of RBC units transfused were associated with early mortality in the absence of grade III-IV aGVHD. A multivariable model for early mortality without
grade III-IV aGVHD identified the earlier era of transplantation (2007-2009) and longer time to platelet engraftment (≥18 days) as independently associated with death (P = .005 and .017, respectively; data not shown).

**RBC Transfusions Associated with Overall Survival**

All surviving patients were followed for at least 2 years post-transplantation (median, 4.7 years; range, 2.0 to 8.5 years). Cumulative survival curves stratified by maximal grade of aGVHD and by numbers of RBC and platelet units transfused between day −7 and day +27 (and before the diagnosis of aGVHD) are shown in Figure 2. Patients with severe aGVHD and those who received more than the median of 5 RBC units or more than the median of 2 platelet transfusions had worse survival (P < .0003, P = .00014, and P = .002, respectively).

In univariate analyses, several factors were significantly associated with worse overall survival (P < .05), including hematocrit on admission, disease risk, HLA mismatch, aGVHD score, and RBC and platelet units transfused (Table 4). There was a trend toward inferior overall survival in patients with increased pretransplantation ferritin levels, but this was not statistically significant (P = .13). Increasing average age of transfused RBC units was inversely associated with overall mortality. In multivariate analysis (excluding aGVHD grade), high-risk disease status and the number of transfused RBC units, but not the number of platelet transfusions, were identified as independently associated with mortality (HR, 1.7 [95% CI, 1.2 to 2.4]; P = .055 and 1.4 [95% CI, 1.0 to 2.0]; P = .054, respectively; Table 4).

**RBC Transfusion Trends Over Time**

We examined the cumulative incidence of first RBC transfusion during the day −7 to day +27 observational window in transplant recipients who were either ABO-matched or ABO-mismatched with their donors. As shown in Figure 3A, 40% of the patients received RBC transfusions during the week before transplantation, and by 2 weeks post-transplantation, the proportion of patients who had received 1 or more RBC transfusions had risen to nearly 90%, with no significant differences in cumulative incidence between these 2 groups. Quantifying the numbers of patients receiving RBC transfusions each week revealed that a significantly higher proportion of patients with an ABO-mismatched donor than those with an ABO-matched donor received RBC transfusions only during the third week post-transplantation (P = .0338; Figure 3B). Compared with myeloablative conditioning regimens, nonmyeloablative conditioning regimens were associated with fewer patients receiving RBC transfusions during weeks 1, 2, and 3 post-transplantation (Figure 3C). Compared with patients with grade 0/I or grade II aGVHD, a significantly larger
fraction of patients who developed grade III-IV aGVHD re-
ceived RBC units each week, except in week 2 (Figure 3D).

Transfusions and Transplantation Outcomes
To analyze possible associations between RBC and plate-
let transfusions and different causes of post-transplantation
mortality, we grouped patients into 4 categories based on the
number of total RBC and platelet units received between day
−7 and day +27 post-transplantation, excluding transfu-
sions received after a diagnosis of aGVHD. Four groups were
defined based on receipt of the median or fewer than the
median number or receipt of more than the median number
of units transfused, resulting in a group of 120 patients who
had received fewer than the median number of both plate-
let and RBC units (group A), a group of 45 patients who
received the median or fewer than the median number of RBC
units but more than the median number of platelet units
(group B), a group of 43 patients who received more than the
median number of RBC units but the median or fewer than
the median number of platelet units (group C), and 114 pa-

Figure 2. Overall survival was associated with aGVHD grade and number of RBC units and platelet units transfused from day −7 to day +27 in Kaplan-Meier analyses. (A) Kaplan-Meier analyses of cumulative survival comparing patients with grade 0/I (blue), II (yellow), and III-IV (maroon) aGVHD. (B) Cumulative survival of patients transfused with >5 RBC units (yellow) versus ≤5 RBC units (blue). (C) Cumulative survival of patients transfused with >2 platelet units (yellow) versus ≤2 platelet units (blue). Plots are truncated at 7 years post-transplantation.

Table 4
Analysis of Factors Potentially Associated with All-Cause Long-Term Mortality

<table>
<thead>
<tr>
<th>Risk Factor (n = 322 Unless Noted Otherwise)</th>
<th>HR</th>
<th>95% CI</th>
<th>P Value</th>
<th>Reliability, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate Cox regression analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at BMT (per 10-yr increase)</td>
<td>1.1</td>
<td>1.0-1.2</td>
<td>.21</td>
<td></td>
</tr>
<tr>
<td>Transplantation in January 2007-December 2009 versus January 2010-January 2013</td>
<td>1.2</td>
<td>.9-1.6</td>
<td>.24</td>
<td></td>
</tr>
<tr>
<td>KPS score (=80 versus ≥90)</td>
<td>1.2</td>
<td>.8-1.6</td>
<td>.40</td>
<td></td>
</tr>
<tr>
<td>HCT on admission (=25% versus ≥25%)</td>
<td>1.5</td>
<td>1.0-2.2</td>
<td>.028</td>
<td></td>
</tr>
<tr>
<td>Disease risk (low, intermediate, high)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate versus low</td>
<td>1.2</td>
<td>.8-1.9</td>
<td>.275</td>
<td>.018</td>
</tr>
<tr>
<td>High versus low</td>
<td>1.9</td>
<td>1.4-2.7</td>
<td>.0002</td>
<td></td>
</tr>
<tr>
<td>Female to male versus other combinations</td>
<td>.8</td>
<td>.6-1.2</td>
<td>.403</td>
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</tr>
<tr>
<td>HLA match</td>
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<td></td>
</tr>
<tr>
<td>10/10 unrelated donor versus 10/10 sibling</td>
<td>1.6</td>
<td>1.0-2.7</td>
<td>.06</td>
<td></td>
</tr>
<tr>
<td>&lt;10/10 match versus 10/10 sibling</td>
<td>2.2</td>
<td>1.3-3.9</td>
<td>.005</td>
<td></td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td></td>
<td></td>
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<tr>
<td>Reduced intensity versus nonmyeloablative</td>
<td>1.1</td>
<td>.6-1.8</td>
<td>.845</td>
<td>.585</td>
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<tr>
<td>Myeloablative versus nonmyeloablative</td>
<td>.9</td>
<td>.5-1.6</td>
<td>.692</td>
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<tr>
<td>Immunosuppression, CSA versus FK506</td>
<td>1.0</td>
<td>.7-1.6</td>
<td>.820</td>
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<tr>
<td>aGVHD score</td>
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<td>&lt;.0001</td>
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<tr>
<td>aGVHD grade II versus 0-I</td>
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<td>.8-1.7</td>
<td>.463</td>
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<td>aGVHD grade III-IV versus 0-I</td>
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<td>1.8-3.8</td>
<td>&lt;.0001</td>
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<tr>
<td>Average age of RBC units (10-d increments; n = 288)</td>
<td>.7</td>
<td>.5-9</td>
<td>.0096</td>
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<tr>
<td>Pre-HSCT ferritin: (&gt;1146 versus ≤1146 ng/ml; n = 299)</td>
<td>1.3</td>
<td>.9-1.8</td>
<td>.13</td>
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<tr>
<td>Transfused RBC units (&gt;5 vs. ≤5)</td>
<td>1.8</td>
<td>1.3-2.4</td>
<td>.0002</td>
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<tr>
<td>Transfused platelet units (&gt;2 vs. ≤2)</td>
<td>1.6</td>
<td>1.2-2.2</td>
<td>.0023</td>
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</tbody>
</table>

Multivariate Cox regression analysis

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>HR</th>
<th>95% CI</th>
<th>P Value</th>
<th>Reliability, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT on admission (=25% versus ≥25%)</td>
<td>1.2</td>
<td>.8-1.8</td>
<td>.391</td>
<td>16</td>
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<tr>
<td>Disease risk (low, intermediate, high)</td>
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<td>.018</td>
<td>79</td>
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<tr>
<td>Intermediate versus low</td>
<td>1.3</td>
<td>.8-1.9</td>
<td>.260</td>
<td></td>
</tr>
<tr>
<td>High versus low</td>
<td>1.7</td>
<td>1.2-2.4</td>
<td>.005</td>
<td></td>
</tr>
<tr>
<td>HLA match</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/10 unrelated donor versus 10/10 sibling</td>
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<td>.9-1.8</td>
<td>.190</td>
<td>.413</td>
</tr>
<tr>
<td>&lt;10/10 match versus 10/10 sibling</td>
<td>1.2</td>
<td>.8-1.9</td>
<td>.392</td>
<td>25</td>
</tr>
<tr>
<td>Transfused RBC units (&gt;5 versus ≤5)</td>
<td>1.4</td>
<td>1.0-2.0</td>
<td>.054</td>
<td>71</td>
</tr>
<tr>
<td>Transfused platelet units (&gt;2 vs. ≤2)</td>
<td>1.2</td>
<td>.9-1.7</td>
<td>.250</td>
<td>34</td>
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</table>
tients who received more than the median number of both RBC and platelet units (group D). Mortality was lowest for the groups with fewer RBC transfusions (group A, 43%; group B, 44%), and greatest for the group with more RBC and platelet transfusions (group D, 66%; \( P = .006 \), Fisher’s exact test compared with group A; Figure 4). Comparisons of the rates of death attributed to relapse, GVHD, organ failure, and secondary malignancy in groups B, C, and D were not statistically significant from those in group A. Only the rate of infection-related deaths was significantly higher in group B than in

<table>
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<tr>
<th>Group, Total N</th>
<th>A, 120</th>
<th>B, 45</th>
<th>C, 43</th>
<th>D, 114</th>
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<tr>
<td>Mortality N (%)</td>
<td>52 (43%)</td>
<td>20 (44%)</td>
<td>23 (53%)</td>
<td>75 (66%)</td>
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<tr>
<td>RBC units</td>
<td>less</td>
<td>less</td>
<td>more</td>
<td>more</td>
</tr>
<tr>
<td>Platelet units</td>
<td>less</td>
<td>more</td>
<td>less</td>
<td>more</td>
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</tbody>
</table>

Figure 3. Weekly frequencies of patients receiving RBC transfusions patients differed based on patient characteristics and aGVHD severity. (A) Cumulative incidence of patients who received RBC transfusions as grouped by ABO match between transplant recipient and donor. (B-D) Percentages of patients receiving RBC transfusions each week (−1 week to +4 weeks post-transplantation) as grouped by ABO match between transplant recipient and donor (B), conditioning regimen intensity (NMA, nonmyeloablative; RIC, reduced intensity conditioning; MAC, myeloablative conditioning) (C), and aGVHD severity (D). *\( P < .05 \); **\( P < .01 \); ***\( P < .001 \).

Figure 4. Causes of death in 4 patient groups based on RBC and platelet transfusions. Groups that received more RBC transfusions had higher frequencies of GVHD-related mortality. Patients were grouped based on the number of total RBC and platelet units received from day −7 to +27 post-transplantation, excluding those received after a diagnosis of aGVHD (>5 versus ≤5 RBC units, and >2 versus ≤2 platelet units). Pie chart sizes are proportional to the percent mortality in each group.
group A (25% versus 6%; \( P = 0.03 \)). Of note, patients who died of organ failure were not overrepresented among groups that received more transfusions, indicating that the numbers of RBC and platelet units transfused in the peritransplantation period were not simply surrogates for greater comorbidities in sicker patients.

**DISCUSSION**

To our knowledge, this study is the first published demonstration of a significant association between the number of RBC units transfused during the pretransplantation and post-transplantation periods with the incidence of severe aGVHD and overall survival in patients undergoing allo-HSCT. In both univariable and multivariable Cox regression analyses, we found significant and robust associations between the number of RBC units transfused (but not platelet units) and the development of grade III-IV aGVHD as well as with overall survival. The use of bootstrapping in the multivariable models allowed for determinations of reliability for these associations (Tables 3 and 4). A recent study in patients with severe aplastic anemia found that the number of pretransplantation RBC transfusions was associated with aGVHD and overall mortality after allo-HSCT, which the authors suggested was attributable to pretransplantation iron overload in the high-transfusion group [42]. In contrast, we excluded patients with nonmalignant hematologic conditions from our study. We analyzed pretransplantation serum ferritin as a surrogate for transfusion history, given that accurate records of transfusions before admission for the conditioning regimen were not available for a patient population from a catchment area across the southeastern states. We did not find an association between increased pretransplantation serum ferritin level and grade III-IV GVHD. As reported in other studies [20-23], we found a trend toward inferior overall survival among patients with higher pretransplantation ferritin levels, although this was not statistically significant in our patient population. The differences in association between pretransplantation ferritin level and GVHD in various clinical studies may be related to differences in patient disease groups and conditioning regimens [22]. Our results suggest that the increased risk of aGVHD in patients who received more RBC transfusions is not accounted for by increased serum ferritin levels, although this is not conclusive owing to a lack of lifetime transfusion records for these patients, which is a limitation of this study.

This retrospective study has several other limitations. First, we restricted our observational time frame for RBC transfusion data to a period during which allo-HSCT recipients were being treated exclusively at the transplantation center (from day -7 pretransplantation to day +27 post-transplantation), and received all transfused units from a single source. Second, to avoid the effects of GVHD-associated gastrointestinal blood loss on transfusion, we only considered transfusions performed before the initial diagnosis of aGVHD, censoring transfusions performed after a diagnosis of aGVHD from the analysis. Finally, aGVHD may be associated with the underlying anemia, such that RBC transfusions are a surrogate measure of anemia in the peritransplantation period. A recent study in immune-incompetent preterm infants demonstrated that severe anemia, but not RBC transfusion, was independently associated with a 6-fold increased risk of necrotizing enterocolitis, an inflammatory and necrotic condition of the intestines [43].

No consistent association between increased aGVHD and ABO mismatch between allo-HSCT donors and recipients has been demonstrated. ABO minor mismatch was associated with increased nonrelapse mortality in 1 study, but there was only a nonsignificant trend toward increased risk for grade II-IV aGVHD [14]. That same study identified a significant association of ABO minor mismatch with grade II-IV aGVHD among the subset of patients who received bone marrow grafts [14]. In another study, ABO incompatibility was not associated with grade II-IV aGVHD in nonmyeloablative allo-HSCT recipients [15]. The mechanism by which transfused RBCs might increase the risk of severe acute GVHD is unknown. Although some RBCs may express trace amounts of major histocompatibility complex class I molecules in the form of Bennett–Goodspeed antigen [44], as a general principle, platelets, but not RBCs, express major histocompatibility complex I [44,45]. Both RBCs and platelets also express minor histocompatibility antigens, with more than 340 known RBC blood group antigens and 33 known platelet alloantigens [46,47]. Minor antigens on donor RBCs enter both class I (cross-presentation) and class II (indirect presentation) pathways [48,49], and thus it is possible that alloantigens present on transfused RBCs may be potently presented by recipient APCs leading to donor T cell activation. Using mouse BMT models, we have demonstrated that interactions between activated donor antigen-presenting cells (APCs) and donor T cells occurring in the first week after allo-HSCT regulate the activation and proliferation of donor T cells, including aGVHD activity [30]. An alternative explanation for the association of RBC transfusions with aGVHD is that innate immune activation may result from transfused RBCs that increase costimulatory molecules on recipient APCs [48], leading to increased activation of donor T cells and increased aGVHD in response to host alloantigens presented by recipient APCs. Further studies are needed to distinguish these possible mechanisms. The distinct association of transfused RBCs, but not platelets, with aGVHD is noteworthy. Transfusion of platelets is also known to induce immune activation in some recipients [33]; however, the fine details of this immune activation are distinct from those for RBC transfusion.

Another potential mechanism that could increase the risk of severe aGVHD after RBC transfusion is increased inflammation in the recipient. Preclinical animal models have clearly demonstrated that transfusion of stored RBCs results in systemic inflammation and immune activation [31,32], whereas controlled trials in healthy volunteers did not demonstrate such an effect [50,51]. Another area of great interest in the field of RBC transfusion is whether longer storage time of RBC units is associated with a higher incidence of post-transfusion complications [52]. Neuman et al. [53] reported that older RBC units have decreased nitric oxide-mediated vasodilatation effects on the vasculature of patients with anemia. The authors discussed the possibility that the products synthesized in RBC units during preservation may be deleterious to patients undergoing allo-HSCT. In our present retrospective study, we observed the opposite trend, with increased average storage time of RBC units associated with better survival, and no association with grade III-IV aGVHD. However, the average duration of storage of RBC units may include very fresh RBC units as well as older units, and thus this measure might not provide a comprehensive representation of RBC unit storage times. We also found no association between the maximum storage time for RBC units and survival or severe aGVHD (data not shown). Other possible factors are the sex and age of the individuals donating RBC units, both of which were shown to impact survival of transfusion recipients [54]. We have initiated a prospective clinical study that includes storage time...
of transfused RBC units, sex and age of RBC donors, as well as measures of inflammation and immune cell activation after RBC transfusion in allo-HSCT recipients.

In conclusion, we have shown that allo-HSCT recipients who received more RBC transfusions between day -7 pretransplantation and day +27 post-transplantation had an increased risk of developing severe aGVHD and had worse overall survival. We constructed a model to estimate the risk of developing severe aGVHD according to the cumulative number of transfused RBC units, assuming that (1) the baseline risk of grade III-IV aGVHD is 15%, (2) the risk of early death without aGVHD is 5%, and (3) the incidence of developing grade III-IV aGVHD is increased by 2% for 7 days after each RBC transfusion. These assumptions correspond to the observed rates of grade III-IV aGVHD and of death without grade III-IV aGVHD (Figure 1). The observed incidence of grade III-IV aGVHD increased with each additional RBC unit transfused and corresponded to the predicted risks from this model (Supplementary Figure S2). After analysis of our retrospective institutional data presented here regarding association of RBC transfusions with increased risk of severe aGVHD, the hematocrit transfusion trigger of <27% that was in place for allo-HSCT recipients in this study (who underwent transplantation between January 2007 and January 2013) has now been lowered substantially, to <21%. Prospective studies, such as our recently opened study at Emory University, are warranted to determine causality between RBC transfusions in allo-HSCT recipients and increased risk of severe aGVHD, providing a further rationale for improving transfusion practices for these patients. Such approaches might reduce the number of transfusions by using symptom-driven criteria and limited transfusion thresholds rather than liberal HCT-driven criteria for RBC transfusion, which in turn may reduce the incidence and severity of GVHD.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.01.003.

REFERENCES


