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Absolute Lymphocyte Count Recovery after Allogeneic Hematopoietic Stem Cell Transplantation Predicts Clinical Outcome

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Immune reconstitution is critical for clinical outcome after allogeneic hematopoietic stem cell transplantation (HSCT). To determine the impact of absolute lymphocyte count (ALC) recovery on clinical outcomes, we conducted a retrospective study of 1109 adult patients who underwent a first allogeneic HSCT from 2003 through 2009, excluding patients who died or relapsed before day 30. The median age was 51 years (range, 18 to 74) with 52% undergoing reduced-intensity conditioning and 48% undergoing myeloablative conditioning HSCT with T cell–replete peripheral blood stem cells (93.7%) or marrow (6.4%) grafts. The median follow-up time was 6 years. To determine the threshold value of ALC for survival, the entire cohort was randomly split into a training set and a validation set in a 1:1 ratio, and then a restricted cubic spline smoothing method was applied to obtain relative hazard estimates of the relationship between ALC at 1 month and log hazard of progression-free survival (PFS). Based on this approach, ALC was categorized as $\leq .2 \times 10^9$ cells/L (low) or $>.2 \times 10^9$ cells/L. For patients with low ALC at 1, 2, or 3 months after HSCT, the overall survival (OS) ($P \leq .0001$) and PFS ($P \leq .0002$) were significantly lower and nonrelapse mortality (NRM) ($P \leq .002$) was significantly higher compared with patients with ALC $> .2 \times 10^9$ cells/L at each time point. When patients who had low ALC at 1, 2, *or* 3 months after HSCT were grouped together and compared, their outcomes were inferior to those of patients who had ALC $> .2 \times 10^9$ cells/L at 1, 2, *and* 3 months after HSCT: the 5-year OS for patients with low ALC was 28% versus 46% for patients with ALC $> .2 \times 10^9$ cells/L, $P < .0001$; the 5-year PFS was 21% versus 39%, $P < .0001$, respectively and 5-year NRM was 40% versus 18%, $P < .0001$, respectively. This result remained consistent when other prognostic factors, including occurrence of grade II to IV acute graft-versus-host disease (GVHD), were adjusted for in multivariable Cox models stratified by conditioning intensity: hazard ratio (HR) for OS: 1.52; $P \leq .0001$; for PFS: 1.42; $P = .0008$; and for NRM: 2.4 $P < .0001$ for patients with low ALC. Low ALC was not significantly associated with relapse (HR, 1.01; $P = .92$) in the multivariable model. Low ALC early after HSCT is an independent risk factor for increased NRM and poor survival independent of grade II to IV acute GVHD.

INTRODUCTION

Immune reconstitution is critical for clinical outcome after allogeneic hematopoietic cell transplantation (HSCT). Because donor lymphoid recovery is a robust surrogate of immune reconstitution, rapid lymphocyte recovery is associated with a survival benefit after allogeneic HSCT [1-10]. However, most of these studies are based on small cohort sizes [4,7,10] and its effect on relapse and nonrelapse mortality (NRM) is inconsistent. Several small studies [3-6] found an association between low absolute lymphocyte cell count (ALC) and relapse and NRM, whereas other studies [7,10] found an association between low ALC and NRM, but not with relapse. Furthermore, these studies covered a wide range of post-transplantation assessment

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time points (days 21 to 100 after HSCT) and proposed varying threshold values for defining low ALC (.175 $\times$ 10^9 cells/L to .5 $\times$ 10^9 cells/L).

We have recently reported the impact of white blood cell count (WBC) recovery early after allogeneic HSCT in a large consecutive cohort of patients undergoing HSCT at our institution [11], and we found a significant association between abnormal WBC within 3 months of HSCT and survival outcome. We now report the results of additional analyses assessing the specific impact of early ALC recovery on HSCT outcomes.

MATERIALS AND METHODS

Patients

The study cohort, as previously reported [11], comprised 1109 consecutive adult patients who underwent first peripheral blood or bone marrow allogeneic HSCT with myeloablative (MAC) or reduced-intensity conditioning (RIC) at Dana Farber Cancer Institute/Brigham and Women's Hospital between 2003 and 2009. Patients receiving umbilical cord blood transplantation, haplo-identical transplantation, or transplantation for benign hematologic conditions were excluded. Patients who died or relapsed within 1 month of HSCT were also excluded as ALC at 1 month could not be assessed. All patients provided consent for use of protected health data for research on a protocol approved by the institutional review board of the Dana-Farber/Harvard Cancer Center. Demographic, clinical, and laboratory data as well as HSCT outcomes were retrieved from our comprehensive institutional transplantation database.

Attainment of ALC Data after HSCT

The ALC at or nearest (within 1 week) the set time points of the study were retrieved from complete blood counts drawn as part of routine clinical care after transplantation. In cases where multiple entries are present, the ALC on the day closest to the set time point is used.

Transplantation

Patients underwent transplantation on a variety of investigational protocols and treatment plans. MAC regimens consisted mostly of cyclophosphamide (3600 mg/m^2 or 120 mg/kg) plus total body irradiation (1400 cGy in 7 fractions), or intravenous busulfan (12.8 mg/kg) plus cyclophosphamide (3600 mg/m^2). RIC regimens consisted primarily of fludarabine (120 mg/m^2) plus intravenous low-dose busulfan (3.2 to 6.4 mg/kg). A small number of patients (<3%) received antithymocyte globulin (ATG) as part of conditioning. Patients received bone marrow or filgrastim-mobilized peripheral blood stem cells from HLA-matched or mismatched, related or unrelated donors. Graft-versus-host disease (GVHD) prophylaxis consisted primarily of a calcineurin inhibitor (cyclosporine or tacrolimus) combined with methotrexate, with or without sirolimus (Table 1). Based on the clinical protocols, filgrastim was usually started on day +12 after MAC transplantation or on day +1 after RIC to hasten neutrophil engraftment, and it was discontinued when the absolute neutrophil counts was over 1000/μL for 2 consecutive days. Supportive care for all patients followed institutional standards.

Chimerism Analysis

In patients undergoing RIC transplantation, day 30 total donor chimerism was assessed from bone marrow aspirates and/or blood approximately 30 days after HSCT. Genotyping was determined by short tandem repeat typing using the ABI Profiler Plus Kit (Applied Biosystems Inc., Foster City, CA) and ABI 310 Genetic Analyzer (Applied Biosystems Inc., Foster City, CA). "Informative" alleles specific to donor or recipient were used for chimerism determination.

Endpoints and Statistical Analysis

The primary objective of the study was to assess the relationship of ALC recovery at 1, 2, and 3 months after HSCT to survival outcome. To rule out the direct influence of relapse on the ALC, a landmark analysis was performed at 1, 2, and 3 months after HSCT excluding relapse or death before each time point. In fact, throughout the analysis, all ALC values that were measured after disease relapse within 3 months of HSCT were censored at each time point. Definitions of overall survival (OS), progression-free survival (PFS), NRM, and relapse are found in previously reports [11,12]. Stratified log-rank test by conditioning intensity was used for comparisons of Kaplan-Meier curves. Cumulative incidences for nonrelapse death and relapse with or without death were estimated reflecting time to relapse and time to nonrelapse death respectively as competing risks. Gray test [13] was used for comparison of cumulative incidence curves. Multivariable proportional hazards models stratified by conditioning intensity were constructed to examine the effect of ALC after adjusting for other potential prognostic factors that are detailed in Table 1, with WBC risk score after HSCT [11] and occurrence of grade II to IV acute GVHD as a time-dependent variable. The proportional hazards assumption for each variable was tested and interaction terms were examined. The linearity assumption for continuous variables was examined using restricted cubic spline estimates of the relationship between the continuous variable and log relative hazard [14], and the cutoff points of these variables were based on the change of the log relative hazards. In particular, age was dichotomized as $\geq$40 versus <40 for MAC and $\geq$60 versus <60 in RIC patients. CD34 cells/kg were categorized as $\leq 4 \times 10^6$, 4 to 15 $\times$ 10^6, and $>15 \times 10^6$ cells/kg. For factors associated with low ALC, multivariable logistic regression analysis was utilized using a backward elimination approach. All *P*-values are 2-sided. Considering multiple comparisons, the significance level was set to .01 for the primary hypothesis but to .05 for secondary analyses. All calculations were done using SAS 9.3 (SAS Institute Inc, Cary, NC), and R version 2.13.2 (the CRAN project).

RESULTS

Patient Characteristics

The baseline characteristics of the 1109 patients are shown in Table 1. The median age was 51 years (range, 18 to 74). The median follow-up time among surviving patients was 6 years (range, 2.5 to 9.8). Fifty-two percent of patients received RIC and 48% received MAC. Seventy-seven percent of patients were Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1 at baseline. Forty-one percent of patients underwent transplantation from HLA-matched related donors, 51% underwent transplantation from HLA-matched unrelated donors, and 8.5% received HLA-mismatched transplantation. Ninety-four percent of patients received stem cells from granulocyte colony–stimulating factor–mobilized peripheral blood. The distribution of disease risk index, which was created based on disease and disease status at transplantation [12], was 16% for low, 53% intermediate, 27% for high, and 3.3% for very high risk.

ALC after HSCT and Threshold Value

The distribution of ALC (excluding values after disease relapse) at 1, 2, and 3 months after HSCT is shown in Figure 1A. The median ALC at these 3 time points was between .64 and .7 $\times$ 10^9 cells/L (interquartile range, .41 to 1.1), and 32% to 36% of patients had an ALC $\leq$ .5 $\times$ 10^9 cells/L during this period. Because the lower cutoff value for normal reference ALC (.5 $\times$ 10^9 cells/L) may not be the most clinically relevant threshold in this patient population, we randomly split the entire cohort into a training set and a validation set in a 1:1 ratio stratified by conditioning intensity, and then we applied a restricted cubic spline smoothing method [14] to obtain relative hazard estimates of the relationship between ALC at 1 month and log hazard of PFS.

The restricted cubic spline curves suggest a sharp decrease in relative hazard initially between 0 and .5 $\times$ 10^9 cells/L, locating the first knot around .2 $\times$ 10^9 cells/L, and then a plateau (Figure S1). We then calculated hazard ratios (HR) of PFS by intervals of 0 to $\leq$.2, .2 to $\leq$.3, .3 to $\leq$.4, .4 to $\leq$.5, .5 to $\leq$2.6 (normal range, reference group), and >2.6 $\times$ 10^9 cells/L (Figure 1B). In Figure 1B, the HR for the interval 0 to $\leq$.2 $\times$ 10^9 cells/L was significantly higher ($P < .001$) than the reference group in both the training and validation sets. Although HRs for intervals of .2 to $\leq$.3, .3 to $\leq$.4, and .4 to $\leq$.5 were higher than the reference group, none of these intervals was significantly high. To facilitate the practical use of ALC data, we thus propose the ALC level by 0 to $\leq$.2 $\times$ 10^9 cells/L versus >.2 $\times$ 10^9 cells/L. Using this cutoff value, 7.5%, 6.4%, and 5.5% of patients had low ALC at 1, 2, or 3 months,