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Early Natural Killer Cell Reconstitution Predicts Overall Survival in T Cell–Replete Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Early immune reconstitution plays a critical role in clinical outcome after allogeneic hematopoietic stem cell transplantation (HSCT). Natural killer (NK) cells are the first lymphocytes to recover after transplantation and are considered powerful effector cells in HSCT. We aimed to evaluate the clinical impact of early NK cell recovery in T cell–replete transplant recipients. Immune reconstitution was studied in 298 adult patients undergoing HSCT for acute myeloid leukemia, acute lymphoblastic leukemia, and myelodysplastic syndrome from 2005 to 2013. In multivariate analysis NK cell numbers on day 30 (NK30) > 150 cells/μL were independently associated with superior overall survival (hazard ratio, .79; 95% confidence interval, .66 to .95; $P = .01$). Cumulative incidence analyses showed that patients with NK30 > 150 cells/μL had significantly less transplant-related mortality (TRM), $P = .01$. Patients with NK30 > 150 cells/μL experienced significantly lower numbers of life-threatening bacterial infections as well as viral infections, including cytomegalovirus. No association was observed in relation to relapse. These results suggest an independent protective effect of high early NK cell reconstitution on TRM that translates into improved overall survival after T cell–replete HSCT.

INTRODUCTION

Although allogeneic hematopoietic stem cell transplantation (HSCT) has improved outcomes for malignant blood disorders considerably during the last decades, it is still a procedure associated with great morbidity and mortality [1]. This is partially because of recurrence from the malignant disease and treatment-related side effects in terms of toxicity, graft-versus-host disease (GVHD), and susceptibility to infections during lymphopenia after transplantation.

Several studies have demonstrated improved survival and lower transplant-related mortality (TRM) in patients with high overall lymphocyte recovery after HSCT [2-5]. These studies, however, did not include enumeration of lymphocyte subtypes. Because natural killer (NK) cells are the first lymphocyte to recover after transplantation [6], it has been suggested that early absolute lymphocyte counts (ALCs) serve as a surrogate marker for NK cell recovery [3,4].

Evidence of NK cells as important effector cells in HSCT in terms of antileukemic and anti-infectious effects is increasing [7,8]. Allogeneic antitumor effects are mediated through killer cell immunoglobulin-like receptors (KIRs) and MHC mismatches between the donor and the recipient, and NK cells mediate lysis of "stressed," infected, or malignant cells through a series of non–MHC-specific activating receptor such as NK receptors and natural cytotoxicity receptors [9].

The impact of NK cells in HSCT has mainly been demonstrated in HLA-mismatched haploidentical transplants with in vivo or ex vivo T cell depletion [10-12]. Mixed outcome results have been reported in HLA-matched transplants [13-15]. Savani et al. [15] showed that high early NK cell reconstitution after HSCT was associated with less acute GVHD (aGVHD), TRM and relapse together with improved survival in 54 acute myeloid leukemia (AML) patients receiving T cell–depleted grafts. Patients with high NK cell recovery, however, did experience more chronic GVHD. In 2008 Dunbar et al. [14] analyzed 167 transplant patients treated with reduced-intensity conditioning and antithymocyte globulin and demonstrated less relapse rates and improved survival in patients recovering with high numbers of NK cells after HSCT;

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this study did not find any difference in the incidence of GVHD. Buhlman et al. [13] was the only group to investigate T cell–replete transplantations and found that high NK cell numbers late after transplantation (days 90 and 180) were associated with less TRM in 345 transplant patients. Therefore, the purpose of this study was to investigate the impact of early NK cell reconstitution on overall survival (OS), TRM, and relapse in a large cohort of HLA-matched T cell–replete stem cell recipients.

METHODS

Patients

Two hundred ninety-eight patients treated with HSCT at The Bone Marrow Transplantation Unit, Department of Hematology, Rigshospitalet, Denmark between 2005 and 2013 were included in the study. Only patients with acute leukemia or myelodysplastic syndrome (MDS) who had a lymphocyte subtype analysis 30 days after transplantation were included. All patients had given written consent to use their medical records and blood analyses for research. The median patient age was 55 years (range, 16 to 74); patient and transplant demographics by study groups are displayed in Table 1. Standard-risk disease was defined by European Group for Blood and Marrow Transplantation criteria as early and intermediate risk score and high-risk disease by European Group for Blood and Marrow Transplantation as late risk score.

Donors and Stem Cell Source

Donors were HLA-identical siblings (n = 99); 1 patient had a 10/10 HLA-identical other related donor. One hundred eighty-nine patients had a 10/10 or 9/10 HLA-matched unrelated donor. One patient had an 8/10 HLA-matched unrelated donor. Eight patients had ≥1 antigen mismatched unrelated donors. Stem cell sources were granulocyte colony-stimulating factor stimulated unmanipulated, T cell–replete peripheral blood stem cells or bone marrow.

Table 1
Patient and Transplant Characteristics by Study Groups

	NK30 < 150 (n = 102)	NK30 > 150 (n = 196)	*P*	Total (N = 298)
Median patient age, yr (range)	53 (16-74)	55 (18-73)		55 (16-74)
Disease				
AML	44	121		165
ALL	22	20	.003	42
MDS	36	55		91
Risk score				
Standard risk	97	185	.796	282
High risk	5	11		16
Patient sex				
Male	37	88	.095	173
Female	65	108		125
Donor sex				
Male	49	80	.232	169
Female	53	116		129
Patient–donor sex				
Female–female	25	50		75
Female–male	24	30	.183	54
Male–male	12	38		119
Male–female	41	78		50
Donor type				
Identical sibling	23	76		99
Other related	1	0	.008	1
Matched unrelated	78	120		198
Stem cell source				
BM	32	28	<.001	60
PBSC	70	168		238
Conditioning				
MA	45	62	.03	107
NMA	102	134		191

Comparison of demographics between patients with absolute NK cell counts higher and lower than 150 cells/μL 30 days after transplantation.
ALL indicates acute lymphoblastic leukemia; BM, bone marrow; PBSC, peripheral blood stem cell; MA, myeloablative; NMA, nonmyeloablative.

TRM, GVHD, and Relapse

TRM was defined as death from causes other than relapse. Acute and chronic GVHD was diagnosed and graded from clinical symptoms and biopsies according to the modified Glucksberg-Seattle criteria [16,17]. Relapse was morphologically diagnosed as more than 5% blast cells in the bone marrow or the appearance of extramedullary leukemic lesions.

Conditioning Regimen

Myeloablative regimens were cyclophosphamide 120 mg/kg plus 12 Gy total body irradiation or 12.2 mg/kg busulfan in myeloid diseases; cyclophosphamide was replaced with Etophophos 1800 mg/m^2 in lymphoid diseases. Doses of cyclophosphamide and Busilvex were calculated using adjusted ideal body weight. Nonmyeloablative conditioning was fludarabine 90 mg/m^2 plus 2 Gy total body irradiation; total body irradiation was increased to 4 Gy in MDS patients not previously treated with chemotherapy.

GVHD Prophylaxis

Myeloablative patients received cyclosporine 6.25 mg/kg bid from day −1 combined with short-course methotrexate on day 1 (15 mg/m^2) and days 3, 6, and 11 (10 mg/m^2). Cyclosporine was tapered to a halt by day 180, unless GVHD was present. Nonmyeloablative patients received tacrolimus .06 mg/kg bid combined with mycophenolate mofetil 15 mg/kg bid in related transplants and tid in unrelated transplants. In the absence of GVHD, mycophenolate mofetil was tapered to a halt by day 27 in related transplants and by day 96 in unrelated transplants, and tacrolimus had a planned tapering to zero by day 180. aGVHD was treated with the addition of prednisone 2 mg/kg, and nonresponders were treated with infliximab 10 mg/kg weekly, occasionally supplemented with extracorporeal photopheresis. Chronic GVHD was treated with prednisone 1 mg/kg, supplemented with calcineurin inhibitors, mycophenolate mofetil, sirolimus, or extracorporeal photopheresis at the discretion of the treating physician.

Patient Samples and Flow Cytometry Analyses

Peripheral blood samples were systematically collected from patients at given time points after transplantation for absolute cell counts of NK cells and T cells; lymphocyte subpopulations were evaluated by flow cytometric analyses with Trucount tubes containing fluorescent beads as an internal standard according to the manufacturer's instructions (BD Biosciences, San Jose, CA). Immunolabeling with CD4 FITC/CD8 PE/CD3 PerCP and CD3 FITC/CD16+CD56 PE/CD45 PerCP from BD Biosciences was used. Samples were analyzed within 24 hours by flow cytometry on FC-500 (Beckman Coulter, Miami, FL) or Navios (Beckman Coulter). CXP and Navios software (Beckman Coulter, Miami, FL) were used for software analyses.

Because of inclusion criteria, sample results by day 30 (range, 20 to 40) after transplantation were available for all 298 included patients. Furthermore, most patients had sample results at day 60 (249/293 [85%]), day 90 (229/281 [81%]), and day 180 (217/260 [83%]). The ALC was available for all 298 patients by day 30 after transplantation.

Endpoint and Statistics

The primary aim of this study was to assess the correlation of absolute NK cell numbers at day 30 (NK30) after transplantation to OS, TRM, and relapse. Estimated OS was analyzed by univariate analysis with the Kaplan-Meier method using the log rank test. Cox models with variables entered as categorical variables were used for multivariable survival analysis. Variables statistically significant in univariate analyses were included in the multivariate analysis. Cumulative incidence rates of TRM, GVHD, and relapse with competing events were compared by Gray's test. Differences between categorical and continuous variables were determined by the chi-square test and Student's *t*-test, respectively. Logistic regression models were used for multivariable analyses of categorical variables. Linear correlation between to variables was analyzed by Pearson correlation. All *P* values were 2-sided and considered statistically significant at <.05. For statistical analyses, SPSS (IBM, New York, NY) and R version 3.2.0 (R Foundation for Statistical Computing, Vienna, Austria) combined with the EZR platform [18] were used.

On the basis of previously published data [13,15,19], cut-off values were set at 150 cells/μL for NK cells and 200 cells/μL for CD4 cells at all time points after transplantation. The terms used for all time points were, exemplified by day 30, NK30 < 150/NK30 > 150 and CD4·30 < 200/CD4·30 > 200. In addition, NK, CD4, and CD8 cells were dichotomized at the median value at all time points after HSCT (NKmedian, CD4median, and CD8median).

RESULTS

After a median follow-up of 4.1 years (range, 1.3 to 10.2), of 298 patients, 167 (56%) were alive, 64 (21%) died from relapse, and 67 (23%) died from TRM. All grades of aGVHD were experienced by 127 patients (43%), and 93 of 298