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Lymphocyte Recovery Is a Major Determinant of Outcome after Matched Unrelated Myeloablative Transplantation for Myelogenous Malignancies

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Abstract

A higher absolute lymphocyte count 1 month (LC30) after allogeneic hematopoietic stem cell transplantation (HSCT) is associated with better outcome in patients transplanted from a matched sibling. We studied 102 SCT patients with unrelated donor and matched unrelated donors and the relationship between LC30 and outcome in patients with myelogenous leukemia. Conditioning was myeloablative using cyclophosphamide (Cy) with busulfan (Bu; n = 61) or total body irradiation (TBI; n = 41). LC30 was low ($<0.2 \times 10^9$/L) in 18 patients, intermediate ($0.2\text{-}1.0 \times 10^9$L) in 67, and high ($>1.0 \times 10^9$/L) in 17 patients. In multivariate analysis, independent factors associated with high relapse-free survival (RFS) were high LC30, high CD34 cell-dose, and absence of acute graft-versus-host disease (aGVHD) grades II-IV. When analyzed as a continuous variable in multivariate analysis, a higher LC30 was associated with a lower transplant-related mortality (TRM; relative hazard [RH] = 0.87, $P<.05$), higher relapse-free survival (RH = 3.42, $P=.036$), and improved survival (RH = 4.53, $P=.016$, excluding GVHD). In patients with high, intermediate, and low LC30, overall survival (OS) was 91% versus 60%, versus 36% ($P=.02$ and .001, respectively). This significant relationship was maintained in patients who did not develop GVHD by day 30. Significant risk factors to develop low LC30 was chronic myelogenous leukemia (CML; hazard ratio [HR] 0.73, $P=.001$), prophylaxis with granulocyte colony-stimulating factor (G-CSF; HR 0.81, $P=.02$) and aGVHD (HR 0.84, $P=.05$). These results indicate that LC30 is an independent prognostic factor for transplant outcome in matched unrelated SCT for myelogenous malignancies.

Keywords

Hematopoietic stem cell transplantation (HSCT); Lymphocytes; Relapse-free survival (RFS); Graft-versus-host disease (GVHD)

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) can cure malignant blood disorders. Repopulating lymphocytes from the donor attack residual tumor cells in the early posttransplant phase and thereby prevent relapse, but, at the same time, limit success of the treatment by causing graft-versus-host disease (GVHD). A slow recovery of the lymphocyte count as a predictor of increased risk of relapse was first proposed in patients treated with myeloablative (MA) conditioning who received HLA-identical sibling grafts as treatment for acute myelogenous leukemia (AML) [1]. In subsequent studies, a low absolute lymphocyte count on day 30 (LC30) predicted worse outcome after HLA-identical sibling transplants receiving both T cell-depleted and unmanipulated grafts [2-7].

Natural killer (NK) cells, which mediate cytotoxicity without prior sensitization, are the first cells to recover in the early posttransplant period [8-10]. Indeed, in haploidentical T cell-depleted transplants, NK-killer cell immunoglobulin-like receptor (KIR) incompatibility reduces the risk of relapse in myelogenous but not lymphogenous malignancies [11]. Similarly, NK cells as the dominant population in the LC30 were recently found to improve transplant outcome in chronic myelogenous leukemia (CML) and AML, but not acute lymphoblastic leukemia (ALL) [6,7].

Today, half of all stem cells transplants (SCTs) are performed using grafts from matched unrelated donors (MUD). Compared to HLA identical sibling transplants, MUD transplants are associated with a higher rate of transplant-related mortality (TRM). The main reasons for this are higher frequencies of rejection and acute GVHD (aGVHD) and an increased incidence of infections because of prolonged immunosuppression.

Taking advantage of the simplicity and reproducibility of the LC30 measurements as a surrogate for NK cell recovery [6], we sought to determine whether prompt lymphocyte recovery also predicted outcome in MUD transplants. We retrospectively analyzed the predictive role of LC30 in a cohort of 102 patients undergoing MUD SCT after MA conditioning. The results indicate that LC30 is a powerful predictor of transplant outcome in myelogenous malignancies.

MATERIAL AND METHODS

Patients

One hundred two patients with myelogenous leukemia receiving MA conditioning and HSCT from a HLA-A, -B, and -DR MUD were included in the study. All patients were transplanted between October 1996 and January 2007, at the Karolinska University Hospital, Stockholm, Sweden. A majority (53%) of the patients had AML, whereas 38 had CML and 10 patients had myelodysplastic syndrome (MDS). The median age was 37 years (range: 0.5-58 years). Patients were considered low risk if they were in first complete remission or chronic phase (CR1/CP1), whereas all others were considered high risk. There were 55 (54%) low-risk and 47 high-risk patients. Patient and donor demographics are displayed in Table 1. The study was approved by the ethical committee and performed in accordance with the declaration of Helsinki.

Donors

There were 58 male and 41 female donors with a median age of 36 years (19-54 years). For 3 transplants, the sex of the donor was unknown. A female donor to a male recipient was present in 12 cases.

HLA-typing

HLA class-II typing was performed using polymerase chain reaction (PCR) amplification with sequence-specific primers (PCR-SSP) [12]. Before 1997, HLA class I-typing was performed by serologic methods. Since 1997, we used PCR-SSP also for HLA class I-typing, initially with low resolution and from 1999 with high resolution. All patients have recently been retrospectively retyped using PCR-SSP with allele level resolution for both HLA class I and II antigens [13]. All patient and donor pairs were HLA-A, -B, and -DR identical. However, an HLA-C mismatch occurred in 32 cases (24 antigen mismatch and 8 allele mismatch). Among the HLA-C mismatched pairs, 17 were KIR mismatched, 12 were KIR matched, and 3 were unknown.

Conditioning

All patients received conventional MA conditioning with cyclophosphamide (Cy; total dose 120 mg/kg) in combination with busulfan (Bu; total dose 16 mg/kg) (n = 61), 10 Gy single-dose total body irradiation (TBI; n = 22), or 12 Gy fractionated TBI (n = 19) [14]. All patients received anti-T cell antibodies during conditioning [15]. Most patients received rabbit antithymocyte globuline (ATG, Thymoglobulin® n = 82, Genzyme, Cambridge, MA), whereas 17 patients received OKT-3 and 3 patients were given Campath (Genzyme). The last dose of ATG was given on the day before (day −1) graft infusion [14].

GVHD prophylaxis

GVHD prophylaxis consisted of cyclosporine (CsA) in combination with 4 doses of methotrexate (MTX; n = 97) [16,17], prednisolone (n = 3), or mycophenolate mofetil [10] (MMF; n = 2). During the first month, the blood CsA levels were kept at 200 to 300 ng/mL [18]. In the absence of GVHD, CsA was discontinued after 6 months.

Stem-Cell Source

A bone marrow (BM) graft was given to 44 (43%) patients, wheres 58 patients received peripheral blood stem cells (PBSC) [19]. Nucleated (NC), $CD34^1$ and $CD3^1$ cell doses are displayed in Table 1.

Supportive Care and Treatment of GVHD

Granulocyte colony-stimulating factor (G-CSF) was given to 63 (62%) patients after HSCT until neutrophil engraftment ($>0.5 \times 10^9$/L) [20]. aGVHD and chronic GVHD (cGVHD) was diagnosed on the basis of clinical symptoms and/or biopsies (skin, liver, gastrointestinal [GI] tract, or oral mucosa) according to standard criteria and treated as previously described [21-23].

Definitions

Engraftment was defined as stable absolute neutrophil counts (ANC) $>0.5 \times 10^9$/L for 3 consecutive days and platelet engraftment as platelet counts $>50 \times 10^9$/L for 7 consecutive days without transfusions.

Statistics

The analysis was performed in January 2008. The probabilities of overall survival (OS) and relapse-free survival (RFS) were estimated using the method developed by Kaplan-Meier and compared with the log-rank test [24]. The incidence of GVHD, TRM, and relapse was estimated nonparametrically. Patients were censored at the time of death, relapse, or last follow-up. Relapse and nonrelapse mortality (NRM) are competing events. Their incidence rates were estimated using a nonparametric estimator of cumulative incidence curves [25].

Predictive analyses for GVHD, TRM, and relapse were based on the proportional hazard model for subdistribution of competing risk. Univariate and multivariate analyses were then performed using Gray's test and the proportional subdistribution hazard regression model developed by Fine and Gray [26]. A stepwise backward procedure was used to construct a set of independent predictors for each endpoint. All predictors with a *P*-value below .10 were considered and sequentially removed if the *P*-value in the multiple model was above .05. All tests were 2 sided. The type I error rate was fixed at .05 for factors potentially associated with time-to-event outcomes. Factors analyzed in the univariate analysis include patient and donor sex and age, sex-mismatch, diagnoses, disease stage, conditioning, GVHD prophylaxis, stem cell source, G-CSF treatment, CMV serology in patients and donors, nucleated and $CD34^+$ cell dose/kg, and GVHD. Analyses were performed using the cmprsk package (developed by Gray, June 2001), Splus 6.2 software, and Statistica software. The Mann-Whitney *U* test was used to compare continuous variables, and the χ^2 method was used to compare the distribution of categoric variables.

RESULTS

Engraftment

The median time to neutrophil and platelet engraftment was 16 (range: 10-32) and 17 (range: 9-210) days, respectively. Platelet, hemoglobin, and neutrophil counts on day 30 are shown in Table 2. The distribution of the LC30 is shown in Figure 1. The median LC30 was 0.48 (range: 0.05-2.8 × 10^9/L). We examined the impact on transplant outcome of various cut-off points, including the median lymphocyte count. The median two-thirds (n = 67) with LC30 0.2-1.0 × 10^9/L showed more homogeneous outcomes, whereas the outlying third (LC30<0.2 × 10^9/L in 18 patients and >1.0 × 10^9/L in the remaining 17) had the greatest disparity in outcome. We therefore elected to analyze outcomes according to 3 subgroups: low (0.2 × 10^9/L), intermediate (0.2-1.0 × 10^9/L), and high (1.0 × 10^9/L). Characteristics for patients with an LC30>1.0 × 10^9/L were similar to those of the entire cohort. When analyzed as continuous variables, the leukocyte count correlated with ANC (r = .97, *P*<.001). Hemoglobin values correlated with the leukocyte (r = .54, *P*<.001) and platelet count (r = .27, *P*=.006), as well as with the ANC (r = .53, *P*<.001). However, there was no correlation between LC30 as a continuous variable and the other values.

Relationship between LC30 and Cytokine Levels

Plasma levels of cytokines were measured in 15 subjects between days 12 and 32 posttransplant (total 21 samples). Six patients had a low (<0.2 × 10^9/L) and 9 patients a high (>1.0 × 10^9/L) LC30. Plasma IL-15 was lower in patients with high LC30 (median 32 pg/mL versus 56.5 pg/mL, *P*>.05 log rank sum).

No significant difference in plasma level of IL-7 was seen between low and high LC30 (median 22.6 pg/mL for high LC30 versus 13.9 pg/mL for low LC30, NS). IL-12 was detectable in only 1 patient with low (<0.2 × 10^9/L) LC30 (median 0 pg/mL) and in 5 of 9 patients (median 152 pg/mL) with high (>1.0 × 10^9/L) LC30. IL-2 was detectable at levels similar to an AB serum pool in only 1 patient with low LC 30 and 1 patient with high LC30.

Factors Influencing Transplant Outcome

aGVHD—The cumulative incidence of aGVHD grades II-IV was 38% (95% confidence interval [CI] 29%-47%) and that of grades III-IV GVHD was 12% (5%-19%). The occurrence of aGVHD grades II-IV was not affected by the donor type, patient age, stem cell source, CD34 cell dose, or type of conditioning.

The only factor associated with a higher risk of aGVHD grades II-IV in univariate analysis was a low LC30, when analyzed as a continuous variable (relative hazard [RH] 0.91, CI: 0.85-0.97, P=0.01) (Table 3).

TRM

An increased risk of TRM was observed in univariate analysis for patients with aGVHD II-IV (RH 13.2, CI: 3.91-44.5, P<.001). Analyzed as continuous variables, a low hemoglobin (0.72, 0.56-0.93, P=.01) and low LC30 (0.85, 0.76-0.96, P=.005) were also associated with increased TRM. In multivariate analyses, aGVHD grades II-IV, a low LC30, and G-CSF administration postgraft were independently associated with an increased risk of TRM (Table 4 and Figure 2a). A low LC30 was still significantly associated with increased TRM if aGVHD was excluded from the multivariate analysis (0.85, 0.76-0.96, P=.005).

RFS

The 5-year RFS for the entire cohort of patients was 56%. Factors associated with a significantly decreased RFS were aGVHD grades II-IV, low LC30, and platelet counts (Table 3). Patients receiving a low CD34 cell dose or G-CSF postgraft also had lower RFS. Multivariate analysis showed that independent factors associated with high RFS were high LC30, high CD34 cell dose, and absence of aGVHD grades II-IV (Table 4). The probability of RFS is shown in Figure 2b. The effect of LC 30 was examined for counts of $<0.2 \times 10^9$/L, $0.2\text{-}1 \times 10^9$/L, and $>1.0 \times 10^9$/L. Patients who had an LC30$<0.2 \times 10^9$ were at a significantly higher risk of treatment failure than those with greater lymphocyte counts (2.53; 1.49-4.31; P>.001).

Survival factors

OS at 5 years for the entire cohort of patients was 61%. Factors identified as significant for inferior OS in univariate analysis were a low CD34 cell dose, low LC30 and platelet counts, and occurrence of aGVHD grades II-IV (Table 3). In multivariate analysis, low CD34 cell dose, low platelet count on day 30, and aGVHD grades II-IV were independently correlated with decreased survival (Table 4). When GVHD was excluded from the multivariate analysis, a low CD34 cell dose (0.96, 0.92-0.99, P=.026) and low LC30 (4.53, 1.32-15.5, P=.016) remained as variables significantly associated with decreased OS. Causes of death in the low ($<0.2 \times 10^9$/L) LC30 group were relapse in 4 (22%), infection in 4 (22%), and GVHD in 3 (17%). In the intermediate LC30 group ($0.2\text{-}1.0 \times 10^9$/L), 12 (18%) patients died of relapse, 8 (12%) of infection, 5 (7%) of GVHD, and 2 of other causes. In the group of patients with LC30$>1.0 \times 10^9$/L only 1 patient died (relapse).

LC30 Is an Independent Variable Influencing Transplant Outcome

Because the LC30 correlated with aGVHD grades II-IV, these patients were analyzed in more detail. Of the 102 patients, 39 developed aGVHD grades II-IV. In the 25 patients who developed ≥ grade II before day 30, mean LC30 was 0.33 versus 0.58 in the 14 patients who developed ≥ grade II after day 30. Figure 2c shows the cumulative incidence of grades II-IV aGVHD in patients with different LC30. aGVHD grades II-IV was significantly more common in patients with a low ($<0.2 \times 10^9$/L) compared to patients with a high ($>1.0 \times 10^9$/L) LC30. Of the patients with an LC30$<0.2 \times 10^9$, 28% (5/18) whereas none of the patients with LC30$>1.0 \times 10^9$ developed severe (grades III-IV) aGVHD (P=.004). Among patients with aGVHD grades II-IV, RFS at 2 years was 22%, 40%, and 100% in patients with LC30$<0.2 \times 10^9$/L (n = 11), $0.2\text{-}1.0 \times 10^9$/L (n = 25) and $>1.0 \times 10^9$/L (n = 3). The corresponding figures for patients with no or grade I aGVHD were 43%, 67%, and 92%. To exclude the possibility that occurrence of aGVHD or its treatment influenced LC30, the RFS

was recalculated, excluding 25 patients who developed aGVHD before day 30. As shown in Figure 2d, excluding aGVHD did not modify the influence of lymphocyte count on RFS.

Risk Factors to Develop Low LC30

We did a risk-factor analysis to identify factors of importance to develop a low LC30. The risk factors included in univariate analysis in addition to those listed in Materials and Methods were AB0 compatibility, Bu compared to TBI, thymoglobuline compared to OKT-3, splenectomy, PBSCs compared to BM grafts. In the univariate analysis, CML, aGVHD, and prophylaxis using G-CSF to promote engraftment were associated with a low LC30. The same factors were also significant in the multivariate analysis (Table 5).

DISCUSSION

There is accumulating data indicating that lymphocyte recovery is a universal factor associated with outcomes of hematologic malignancies after chemotherapy, autologous BM transplantation, and allogeneic SCT between identical siblings [27]. Our study, the first to specifically evaluate unrelated SCT, concords with the general observation that higher lymphocyte counts favor better outcome, because of a lower TRM, less relapse, and less GVHD, and extends this finding to the behavior of unrelated donor lymphocytes recovering in transplant recipients of HLA matched blood and marrow transplants. It could be argued that aGVHD or its treatment with steroids could have affected the lymphocyte count and that the LC30 was only a surrogate for GVHD-related events. In multivariate analysis both LC30 and aGVHD were independent predictors of outcome. However, when aGVHD was excluded from the multivariate analysis LC30 remained an independent variable significantly associated with decreased OS. Furthermore, when the RFS was recalculated, excluding 25 patients who developed aGVHD before day 30, there was no difference in the significant impact of LC30 on RFS.

The mechanism underlying a predictive effect of lymphocyte recovery on outcome is not well defined. It remains possible that LC30 is a surrogate for a lymphocyte subset, and there is evidence from other studies that the LC30 correlated with recovery of NK cells [6,7]. In this retrospective analysis, we did not measure NK cells. Instead, we sought to relate lymphocyte recovery with plasma cytokine levels in the early posttransplant period. Only IL-15 was detectible at levels elevated above the plasma control pool in all samples. We found an inverse correlation between higher IL-15 levels (a growth factor for NK^- and $CD8^+$ T cells) and LC30, consistent with negative feedback from a more rapid NK cell recovery, limiting growth factor production. Because early recovering NK cells are derived from $CD34^+$ progenitors [6,7], the direct relationship between LC30 and CD34 cell dose is also consistent with a predominant NK recovery on day 30. To better define the relationship of lymphocyte recovery with specific lymphocyte subsets and cytokine patterns after SCT more extensive studies will be needed.

Lymphocyte recovery appeared to be independent of the presence or absence of donor-recipient KIR mismatch, indicating that recovery of counts did not relate to NK allogenicity per se. Our material did not allow us to investigate whether a particular donor KIR genotype correlates with high LC30 as reported by Savani et al. [6,7]. However, consistent with that study, the favorable effect of LC30 on relapse was not seen in recipients with ALL transplanted in our center (data not shown). Because ALL cells are less susceptible to NK cytotoxicity than AML cells, this further supports an NK cell-dependent mechanism driving the outcome. Finally, it is possible that the LC30 may simply be a surrogate for the quality of engraftment. However, although the total leukocyte count correlated with both neutrophil and platelet counts as well as with the hemoglobin, no correlation was found between the LC30 and the other variables.

Assuming that LC30 is in some way a biologically relevant predictor of transplant outcome, the possibility of improving results of URD transplants by strategies to increase lymphocyte recovery seems worth exploring. NK cell recovery, for example, might be improved by increasing CD34 count [6,7] (itself a powerful predictor for outcome) [28]. Our data may support studies treating patients with BM boost, mesenchymal stem cells, or evaluating the adoptive transfer of donor NK cells (which do not increase the risk of aGVHD) [29-31]. In addition, risk-adapted strategies could be applied to recipients who failed to achieve an LC30>0.2×10^9/L, for example, selecting such patients for preemptive donor lymphocyte infusions (DLIs) to prevent relapse, intensifying GVHD prophylaxis, and providing longer prophylactic coverage for opportunistic infections.

Finally, we did a risk-factor analysis for low LC30. The most significant factor was CML. The reason for this is unclear because patients with CML generally have a good prognosis after autologous SCT (ASCT).

We have previously reported that G-CSF used to promote engraftment was associated with an increased risk of GVHD and death [32]. Some studies have shown an increased mortality using G-CSF as prophylaxis after ASCT, although contradictory data also exist [33]. Although G-CSF increases ANC, it causes platelet aggregation and prolongs platelet engraftment after ASCT [32]. It may therefore be possible that, although it while promotes the production of ANC, it adversely affects lymphocyte recovery [32]. That GVHD decreases lymphocyte recovery is quite expected, because GVHD has both direct and indirect effects on hematopoiesis by a graft-versus-hematopoietic effect, and also by increasing the risk of infections and toxic complications, leading to hemorrhages and leukocyte consumption. All patients included in this analysis were treated with ATG, OKT3, or Campath. It is possible that treatment with anti-T cell antibodies may delay lymphocyte recovery. However, because all patients were treated equally, it does not explain differences noted between the different groups of patients analyzed here. Lymphocyte recovery was also similar in patients treated with either thymoglobulin or OKT-3.

In conclusion, lymphocyte count early after SCT is 1 of the most universally measured reproducible and powerful predictive factors for outcome. LC30 can therefore readily be included as an outcome variable in analyses of large multicenter databases to further define its prognostic significance in different disease and transplant types. Meanwhile, the predictive power of the finding should stimulate further immunologic laboratory studies to define the mechanism driving lymphocyte recovery and NK cell recovery in particular, with a view to optimizing posttransplant outcome by maximizing immune reconstitution after SCT.

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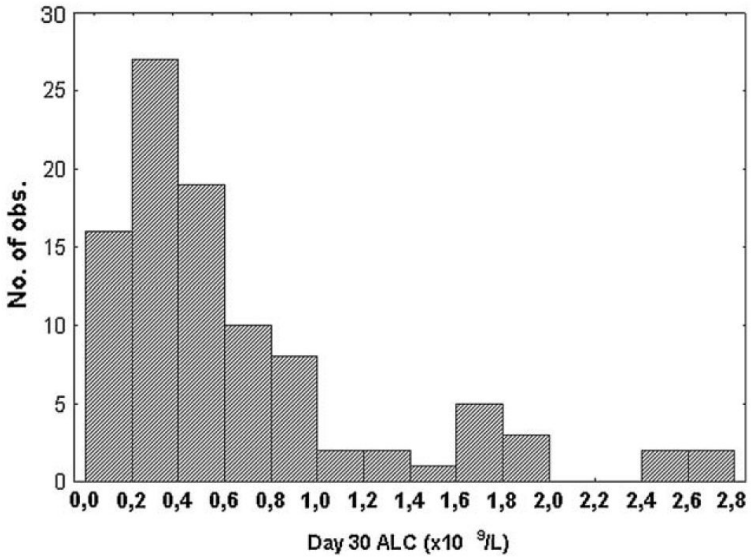

Figure 1.
Distribution of absolute lymphocyte counts at day 30 after unrelated donor HSCT. Figures are given as $\times 10^9$/L.

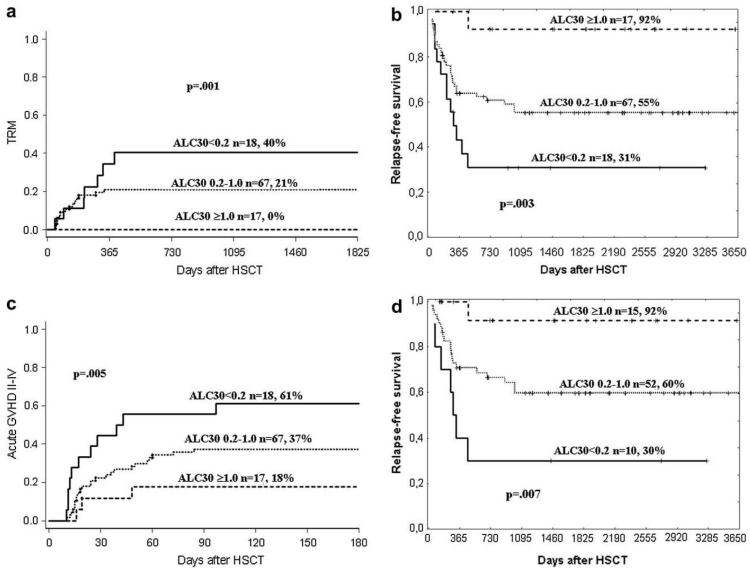

Figure 2.

(a) Cumulative incidence of treatment-related mortality (TRM), (b) Actuarial relapse free survival (RFS) for all patients, (c) Cumulative incidence of aGVHD grades II-IV of patients with an absolute lymphocyte count on day +30 (LC30) $<0.2 \times 10^9$ (solid line), $0.2\text{-}1.0 \times 10^9$ (dotted line) and $\geq 1.0 \times 10^9$ (dashed line). (d) Actuarial RFS for 77 patients who did not develop aGvHD before day 30.

Table 1

Characteristics of Patients and Donors Included in the Study Evaluating Lymphocyte Counts at day 30 after HSCT

HSCT with MUD	**N = 5 , or median (range)**
N =	102
Diagnosis	
AML	54
CML	38
MDS	10
Risk (low/high)	55/47
Age	37 (0-58)
Children (<18 years)	22 (22%)
Sex (M/F)	57/45
Donor age	36 (19-54)
Donor sex (M/F)	58/41
Female donor to Male recipient	12(12%)
Stem cell source (BM/PBSC)	44/58
NC dose ($\times 10^8$/kg)	7.6 (0.6-63.8)
CD34 dose ($\times 10^6$/kg)	6.8 (0.2-56.4)
GVHD prophylaxis	
CsA + MTX	97
Other	5
Conditioning:	
TBI-based	41
Bu-based	61
G-CSF post-HSCT	63 (62%)

HSCT indicates hematopoietic stem cell transplantation; MUD, matched unrelated donors; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; BM, bone marrow; PBSC, peripheral blood stem cells; NC, nucleated; CsA, cyclosporine; GVHD, graft-versus-host disease; MTX, methotrexate; Bu, busulfan.

Table 2

Levels of Leukocytes, Lymphocytes, Absolute Neutrophils (ANC), Hemoglobin, and Platelets at Day 30 after HSCT Depending on Diagnosis

	AML	CML	MDS	All patients
Leukocytes	5 (0.7-20.1)	3.7(0.7-17.4)*	7.0(0.6-16.4)	4.6 (0.6-20.1)
Lymphocytes	0.58 (0.05-2.8)	0.31 (0.09-l.7)†	0.58 (0.08-2.0)	0.48 (0.05-2.8)
ANC	3.0(0.09-18.1)	2.8(0.5-13.6)	4.9 (0.4-11.1)	2.9 (0.09-18.1)
Hemoglobin	103 (71-140)	98 (69-133)	92 (80-124)	100(69-140)
Platelets	75 (7-374)	74 (8-324)	81 (6-184)	75 (6-374)

AML indicates acute myelogenous leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; HSCT, hematopoietic stem cell transplantation; ANC, absolute neutrophil count.

*$P = .01$ versus AML.

†$P < .001$ versus AML.

Table 3

Results from the Univariate Analysis of Factors Associated with Overall Survival and Relapse-Free Survival after Unrelated Donor HSCT

	Survival			RFS		
	RH	**95% CI**	***P*-value**	**RH**	**95% CI**	***P*-Value**
LCC *	1.04	0.54-2.03	.90	0.91	0.46-1.81	.78
ALC *	0.35	0.15-0.85	.02	0.30	0.12-0.72	.007
ANC *	1.20	0.59-2.44	.61	1.23	0.71-2.14	.46
HB *	0.83	0.68-1.01	.06	0.84	0.70-1.02	.08
Platelet *	0.93	0.88-0.99	.022	0.95	0.90-0.99	.04
Patient age *	1.13	0.92-1.37	.24	1.10	0.91-1.33	.34
Donor age *	0.91	0.59-1.40	.66	0.92	0.61-1.38	.68
NC dose *	1.06	0.82-1.37	.66	1.02	0.79-1.32	.87
CD34 dose *	1.04	0.99-1.08	.07	1.04	1.00-1.09	<.05
Low risk versus high risk	1.42	0.76-2.66	.28	1.30	0.71-2.38	.40
Acute leukemia versus all others	1.60	0.84-3.06	.15	1.43	0.78-2.63	.25
TBI versus Busulfan	0.88	0.47-1.64	.68	0.86	0.47-01.58	.63
aGVHD 0-1 versus aGVHD II-IV	3.90	2.04-7.44	.00004	3.35	1.83-6.16	.0001
FD to MR versus all other combinations	1.35	0.56-3.26	.50	1.19	0.50-2.81	.70
PBSC versus BM	1.39	0.73-2.66	.32	1.22	0.67-2.24	.53
cGVHD yes/no	1.05	0.50-2.21	.89	0.90	0.44-1.87	.78
G-CSF yes/no	1.95	0.93-4.12	.08	2.05	1.01-4.16	<.05

LCC indicates day 30 leukocyte cell count; ALC, day 30 absolute lymphocyte count; ANC, day 30 absolute neutrophil count; HB, day 30 hemoglobin level; platelets, day 30 platelet count; NC dose, nucleated cell dose in graft ($\times 10^8$/kg), CD34, $CD34^+$ cell dose in graft ($\times 10^6$/kg); low risk, CR1/CP1; high risk; >CR1/CP1; TBI, total body irradiation; aGVHD, acute graft-versus-host disease; FD to MR; female donor to male recipient; BM, bone marrow; PBSC, peripheral blood stem cells; cGVHD; chronic graft-versus-host disease; RFS, relapse-free survival; CI, confidence interval; G-CSF, granulocyte-colony stimulating factor; RH, relative hazard.

*Analyzed as continuous variable.

Table 4

Results from the Multivariate Analysis of Factors Associated with Overall Survival (OS), Relapse-Free Survival (RFS), Treatment-Related Mortality (TRM), and Graft-versus-Host Disease (GVHD) after Unrelated Donor HSCT

	Transplant-Related Mortality		
	RH	**95%CI**	***P*-Value**
aGVHD II-IV	12.3	3.63-41.7	<.001
LC30 *	0.88	0.77-1.00	<.05
G-CSF	3.42	1.21-9.68	.02
Relapse-free survival			
aGVHD II-IV	0.39	0.18-0.82	.014
LC30 *	3.42	1.07-10.9	.036
CD34 dose *	0.95	0.91-0.99	.024
Overall survival			
aGVHD II-IV	0.19	0.09-0.40	<.001
Platelets D +30 *	1.10	1.04-1.17	.006
CD34 dose *	0.94	0.90-0.98	.002

LC30 indicates day 30 absolute lymphocyte count; platelets, day 30 platelet count; CD34 dose, $CD34^+$ cell dose in graft ($\times 10^6$/kg); aGVHD; acute graft-versus-host disease; G-CSF; granulocyte-colony stimulating factor administration postgraft; HSCT, hematopoietic stem cell transplantation.

* Analyzed as continuous variables.

Table 5

Results from the Multivariate Analysis of Factors Associated with low LC30

	RH	95% CI	*P*-Value
CML	0.74	0.34-0.78	0.001
Prophylaxis with G-CSF	0.81	0.68-0.97	0.02
Acute GVHD II-IV (before day 30)	0.84	0.70-1.00	0.05

RH indicates relative hazard; CI, confidence interval; G-CSF, granulocyte-colony stimulating factor; CML, chronic myelogenous leukemia; LC30, day 30 absolute lymphocyte count.