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White Blood Cell Recovery After Allogeneic Hematopoietic Cell Transplantation Predicts Clinical Outcome

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Abstract

To determine whether outcome after allogeneic hematopoietic cell transplantation (HCT) could be estimated by using peripheral white blood cell count (WBC) as a metric that integrates several aspects of HCT recovery, we conducted a retrospective study of 1109 adult patients who underwent first allogeneic HCT from 2003 through 2009. WBC at 1, 2, and 3 months after HCT was categorized as low (<2), normal (2-10), and high (>10×10^9 cells/L). Overall survival (OS) and progression-free survival (PFS) were lower for patients with low or high WBC at 1, 2, or 3 months after HCT (p<0.0001). We developed a predictive 3-group risk model based on the pattern of WBC recovery early after HCT. Five-year OS was 47%, 30%, and 15% (p<0.0001) and 5-year PFS was 39%, 22%, and 14% for patients in the 3 different risk groups (p<0.0001). The pattern of WBC recovery early after HCT provides prognostic information for relapse, non-relapse mortality, progression-free survival, and overall survival. A scoring system based on the trajectory of the WBC in the first 3 months after HCT can effectively stratify patients into 3 groups with different PFS and OS. If validated, this system could be useful in the clinical management of patients after HCT, and to stratify patients enrolled on HCT clinical trials.

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Conflict of Interest Disclosures

The authors have no relevant conflicts of interest to disclose.

AUTHORSHIP CONTRIBUTIONS

H. T. K. designed and performed the research, analyzed the data, and wrote the paper.
D. F. collected data and reviewed the paper.
P. A. collected data, reviewed and wrote the paper.
E. A. collected data and reviewed the paper.
G. K. collected data and reviewed the paper.
C. C. collected data and reviewed the paper.
J. K. collected data and reviewed the paper.
E. P. A. collected data and reviewed the paper.
J. H. A. collected data and reviewed the paper.
R. J. S. collected data and reviewed the paper.
J. R. collected data and reviewed the paper.
V. T. H. collected data, oversaw the data collection team from the database, and wrote on the paper.

Keywords

White blood cell count; Allogeneic transplantation; Prognostic risk group

INTRODUCTION

The success of allogeneic hematopoietic stem cell transplantation (HCT) depends, in large part, on the robust recovery of the lymphoid and myeloid hematopoietic system. While the complexity of immunologic reconstitution is still being unraveled, studies have suggested that early hematopoietic recovery after HCT can be useful prognostic markers for HCT outcome. Early recovery of absolute lymphocyte count (ALC) [1-8], lymphocyte subsets [9-10], absolute monocyte counts [7-8], number of circulating NK cells [11] or invariant natural killer T (iNKT)/T cell ratios [12] have been reported to be associated with survival, non-relapse mortality, relapse, and acute GVHD. Recently, pre-transplantation ALC level was also shown to be associated with relapse and survival [13]. In contrast to leukocyte subsets, the total circulating white blood cell count (WBC) integrates both myeloid and lymphoid elements, and could also be a marker of immunohematopoietic function. Perturbations of the WBC early after HCT could reflect poor stem cell number and function, host microenvironment defect, inflammation, infection, and medication effect. We hypothesized that WBC recovery as a whole, and the patterns of WBC changes early after HCT might provide a simple, yet independent prognostic marker for clinical outcomes after HCT. To test this hypothesis, we conducted a large retrospective analysis of patients undergoing HCT for hematologic malignancies at our institution.

METHODS

Patients

The study cohort was comprised of 1109 consecutive adult patients who underwent first peripheral blood or bone marrow allogeneic HCT with myeloablative or reduced intensity conditioning at Dana Farber Cancer Institute/Brigham and Women's Hospital between 2003 and 2009. Patients receiving umbilical cord blood transplantation, haplo-identical transplant, or transplantation for benign hematologic conditions were excluded. Patients who died or relapsed within one month of HCT were also excluded since WBC 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 HCT outcomes were retrieved from our comprehensive institutional transplantation database.

Attainment of WBC data after HCT

The WBC at or nearest (usually within 1week) the set time points of the study were retrieved from complete blood counts (CBC) drawn as part of routine clinical care after transplantation.

Transplantation

Patients were transplanted on a variety of investigational protocols and treatment plans. Myeloablative conditioning (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). Reduced intensity conditioning (RIC) regimens consisted primarily of fludarabine (120 mg/m^2) plus intravenous low-dose busulfan (3.2-6.4 mg/kg). 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 (G-CSF) was usually started on day+12 after MAC transplantation, or on day +1 after RIC to hasten neutrophil engraftment, and discontinued when the absolute neutrophil counts is 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 HCT. Genotyping was determined by short tandem repeat typing using the ABI Profiler Plus Kit (Applied Biosystems Inc.) and ABI 310 Genetic Analyzer. "Informative" alleles specific to donor or recipient were used for chimerism determination.

Endpoints and Statistical Analysis

The primary goal of the study was to assess the relationship of WBC recovery at 1, 2, and 3 months after HCT to overall survival (OS) and progression-free survival (PFS). To rule out the direct influence of relapse on the WBC, a landmark analysis was performed at 1,2,3 months post HCT excluding relapse or death prior to each time point. In fact, throughout the analysis, all WBC values that were measured after disease relapse within 3 months of HCT were censored at each time point. OS and PFS were previously defined [14]. Log-rank test was used for comparisons of Kaplan-Meier curves. Cumulative incidences for non-relapse death and relapse with or without death were estimated reflecting time to relapse and time to non-relapse death respectively as competing risks. Gray test [15] was used for comparison of cumulative incidence curves. Multivariable proportional hazards models stratified by conditioning intensity were constructed to examine the effect of WBC after adjusting for other potential prognostic factors that are detailed in Table 1 and occurrence of grade II-IV acute GVHD and grade 3 or higher infection within 100 days of HCT. The occurrence of grade II-IV and grade ≥3 infection were included as time dependent variables in multivariable analysis. 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 [16] and the cutoff points of these variables were based on the change of the log relative hazards. In particular, WBC was categorized as 0-<2×10^9 cells/L, 2-<4×10^9 cells/L, and >10×10^9 cells/L and age is dichotomized as ≥40 vs. <40 for MAC and ≥60 vs. <60 in RIC patients. CD34 cells/kg was categorized as ≤4×10^6, 4-15

$\times 10^6$, and $>15 \times 10^6$ cells/kg. Risk factors for low or high WBC were examined using multivariable multinomial logistic regression analysis (i.e., low vs. normal, high vs. normal). All *p*-values are two-sided. A significance level of 0.025 was employed to take multiple comparisons (low vs. normal, high vs. normal WBC) into account. However, when exploring potential risk factors for low or high WBC, a significance level of 0.05 was employed. All calculations were done using SAS 9.3 (SAS Institute Inc, Cary, NC), and Rversion 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 18-74). The median follow up time among surviving patients was 5.1 years (range 1.1-9.6). Fifty-two percent of patients received reduced intensity conditioning (RIC) and 48% received myeloablative conditioning (MAC). Seventy-seven percent of patients were ECOG performance status 0 or 1 at baseline; 41% of patients underwent transplantation from HLA-matched related donors, while 51% were transplanted from HLA-matched unrelated donors, and 8.5% received HLA-mismatched transplantation; 94% of patients received stem cells from G-CSF mobilized peripheral blood. The distribution of disease risk index (DRI), which was created based on disease and disease status at transplantation [14], was 16% for low, 53% intermediate, 27% for high and 3.3% for very high risk.

White Blood Cell Counts After HCT and Threshold Value

The distribution of WBC (excluding values after disease relapse) at 1, 2 and 3 months after HCT is shown in Figure 1-(A). The median WBC at these 3 time points was between 4.4 and 4.8×10^9 cells/L, and 36% - 42% of patients had WBC below 4×10^9 cells/L during this period. This suggests that many patients experience a prolonged period of leukopenia after HCT. Since the lower limit of WBC (4×10^9 cells/L) may not be clinically the most relevant threshold in this patient population, we determined empirically the optimal thresholds by using restricted cubic spline estimates of the relationship between WBC and log relative hazard of overall survival (14). The spline smoothing curve suggests that there is a U-shaped relationship between the level of WBC and hazard of overall survival. To confirm this U-shaped relationship, we plotted hazard ratios of overall survival by intervals of WBC counts (Figure 1-(B)). The hazard ratio of WBC 0-$<2\times10^9$ cells/L relative to the reference group of WBC 4-$<6\times10^9$ cells/L was 1.51 (p=0.01), 1.6(p=0.004), and 1.5 (p=0.04), respectively, at 1,2,3 months after HCT. Similarly, the hazard ratio of WBC $\geq10\times10^9$ cells/L relative to the reference group was 1.76 (p<0.0001), 1.74(p=0.002), and 1.62 (p=0.016), respectively, at 1,2,3 months after HCT. It is noted that the hazard ratio of WBC 2-4×10^9 cells/L to the reference group is close to 1 at each time point (0.91, 1.03, 0.98 at 1,2,3 months after HCT, respectively). Based on this information, we categorized WBC as “low” (0-$<2\times10^9$ cells/L), “normal” (2-10×10^9 cells/L), or “high” ($>10\times10^9$ cells/L). Using this definition, 6-7% of patients had low WBC and 5-10% of patients had high WBC at 1,2, or 3 months post HCT. Overall, 27% of patients had either low or high WBC at some point during the first 3 months of HCT.

Impact of WBC on Survival Outcomes

Regardless of the time point (1, 2 or 3 months after HCT), a low or a high WBC was associated with inferior OS and PFS. Five-year OS was 33%, 45%, 33% for patients with low, normal, or high WBC at 1 month after HCT, respectively (p<0.0001), 35%, 50%, 37% at 2 months after HCT, respectively (p<0.0001), and 41%, 53%, 48% at 3 months after HCT, respectively (p≤0.0025). Five-year PFS was 25%, 38%, 30% for patients with low, normal, or high WBC at 1 month after HCT, respectively (p=0.0001), 27%, 42%, 33% at 2 months after HCT, respectively (p<0.0001), and 37%, 45%, 42% at 3 months after HCT, respectively (p=0.03 for L vs. N, 0.037 for H vs. N) (Figure 2, Table S1). This was true even when RIC and MAC patients were analyzed separately except for MAC at 3 months after HCT. For MAC patients, low or high WBC at 3 months after HCT was not statistically different compared to patients with normal WBC (Table S1).

Pattern of WBC recovery

Since some patients with either low or high WBC at 1 or 2 months after HCT recovered at the subsequent time point(s), we investigated the pattern of WBC recovery and its impact on survival outcome. We noted that many patients developed newly low or high WBC at 2 or 3 months after HCT, and this was an equally high risk of poor outcome as it was for patients whose WBC was low or high at 1 month as well. When each type of WBC trend (i.e., normal to low/high or vice versa) was examined using Cox models stratified by conditioning intensity, we found that patients with high WBC at 1 month who subsequently recovered their WBC to normal at 2 and 3 months after HCT had similar OS compared to patients with sustained normal WBC throughout the 3 month period (n=46, HR=1.05, p=0.84). Also, patients with normal WBC at 1 month who had one time transient low or high WBC during the 3 month period had similar OS compared to patients with sustained normal WBC throughout the 3 month period (n=84, HR=1.1, p=0.54). Thus we combined these groups as a referent group (WBC risk group 0). The remaining patients with first low WBC at 1 or 2 months (WBC risk group 1) or patients with first high WBC at 1 or 2 months after HCT (WBC risk group 2) had poor OS compared to the referent group (Table 2). Patients who died or relapsed after 1 month were scored based on their 1 month WBC value. Likewise, patients who died or relapsed after 2 months of HCT were scored based on their 1 and 2 months WBC values. In addition, patients with missing WBC at 2 months (n=4) and at 3 months after HCT (n=24) were categorized based on two available WBC values. To investigate whether these patients were a random subset of the entire cohort, the OS was compared between these 29 patients and 1080 patients and the OS was similar between these two cohorts (5-year OS 43% vs. 41%, p=0.86).

Based on this classification, the 5-year OS was 47%, 30%, and 15% for WBC risk group 0,1, and 2, respectively (p<0.0001) and the 5-year PFS was 39%, 22%, and 14% for WBC risk group 0,1, and 2, respectively (p<0.0001) (Table 3, Figures 3A and 3B). This result was consistent when other prognostic factors were adjusted for in multivariable Cox models stratified by conditioning intensity (HR 1.64, 3.06 for OS for WBC risk group 1 and 2 respectively, p≤0.0002; HR 1.49, 2.88 for PFS for WBC risk group 1 and 2 respectively, p≤0.0017) (Table 4). Other factors that were significantly associated with OS in the multivariable analysis were age (HR 1.27 for age>=40 in MAC and age>=60 in RIC, p=0.005), ECOG performance status (HR 1.31 for 1 vs 0, p=0.007; HR 1.58 for 2-3 vs 0, p=0.002), disease risk index (HR 1.73 for Intermediate vs. Low, p<0.0001, HR 2.68 for High/very high vs. Low, p<0.0001), occurrence of grade II-IV acute GVHD (HR 1.5, p<0.0001), and any grade ≥3 infection within day 100 (HR 1.89, p<0.0001). Other factors that were significantly associated with PFS in the multivariable analysis were ECOG performance status (HR 1.29 for 1 vs. 0, p=0.007; HR 1.42 for 2-3 vs. 0, p=0.01), disease risk index (HR 1.93 for Intermediate vs. Low, p<0.0001, HR 2.98 for High/very high vs. Low, p<0.0001), year of transplantation (HR 0.95, p=0.03), CD34 cell dose (HR 1.31 for $\leq 4\times10^6$ vs $4\text{-}15\times10^6$ cells/kg, p=0.03) and occurrence of grade ≥3 infection within day 100 (HR 1.71, p<0.0001). Detailed results of the multivariable analysis are provided in Table 4. In multivariable analysis, the occurrence of grade II-IV acute GVHD and day 100 grade ≥3 infection status were included as time dependent variables.

To further evaluate the impact of WBC on cumulative incidences of relapse and NRM, competing risks data analysis was performed. The 5-year cumulative incidence of NRM was 19%, 28%, 37% for WBC risk group 0, 1, and 2, respectively (p=0.008, p=0.0005, respectively), and the 5-year cumulative incidence of relapse was 41%, 51%, and 49% for WBC risk group 0,1, and 2 respectively (p=0.29, p=0.0005, respectively) (Table 3, Figures 3C and 3D). As in the survival outcome, this result was consistent when other prognostic factors were adjusted for in multivariable regression models stratified by conditioning intensity (HR 2.04, p=0.0009; HR 3.23, p<0.0001 for NRM for WBC risk group 1 and 2, respectively; HR 1.27, p=0.13; HR 2.86, p<0.0001 for relapse for WBC risk group 1 and 2, respectively) (Table 4). Other factors that were significantly associated with NRM in the multivariable analysis were age (p=0.036), matched unrelated donor (p=0.001), mismatched donor (p=0.003), ECOG performance status ≥2 (p=0.007), occurrence of grade II-IV acute GVHD (p<0.0001), and any grade ≥3 infection within day 100 (p<0.0001) (Table 4). Other factors that were significant for relapse in the multivariable analysis were matched unrelated donor (p=0.008), disease risk index (p<0.0001), CD34 cell dose (p=0.045), ECOG performance status (p=0.017), year of transplantation (p=0.04), and occurrence of grade II-IV acute GVHD (p=0.02) (Table 4).

Landmark Analysis

To confirm that the WBC risk group was not just a predictor of imminent relapse or NRM, but have prognostic significance for survival outcomes further into the future, we performed a landmark analysis restricted to patients who were alive and relapse-free beyond day 100 of HCT. The 5-year OS was 55%, 40%, 38% for WBC risk group 0,1, and 2, respectively (p≤0.0002) and 5-year PFS was 47%, 29%, 34% for WBC risk group 0,1, and 2, respectively (p≤0.0005) (Figure S1).

Factors affecting abnormal WBC

Because the impact of WBC recovery early after HCT on clinical outcome was statistically significant, we sought to identify factors that affect WBC during the first 3 months after HCT. In multivariable logistic regression analysis, patients receiving MAC were more likely to develop high WBC (OR=2.47 compared to RIC, p<0.0001); sirolimus use as GVHD prophylaxis (OR=0.62, p=0.009) and low CD34 cells infused (OR=0.50 for $< 4\times10^6$ vs

4-15×10^6 cells/kg, p=0.04) were less likely to develop high WBC. For low WBC, patients receiving peripheral blood stem cells were less likely to develop low WBC (OR=0.40, p=0.04). For RIC patients, failure to achieving >=90% day 30 chimerism level was also associated with low WBC (OR=1.71, p=0.039).

We also investigated association of low or high WBC and infection that occurred within 100 days of HCT and found that low or high WBC was associated with viral and fungal infection, but not with bacterial infection (data not shown). Grade 3 or higher viral or fungal infection rate was 19%, 9%, 17% for patients with low, normal, high WBC, respectively (p=0.0002).

DISCUSSION

Recovery of the total WBC after HCT is affected by engraftment kinetics, infection, drug toxicity, persistence or relapse of hematologic malignancy, and marrow microenvironment defects. While much attention has been paid to post transplant metrics such as absolute lymphocyte count, and lymphoid chimerism, the impact of WBC as a whole early after HCT on survival has not been evaluated. We hypothesized that the WBC, as a ubiquitous and standard laboratory metric measured across the first few months after transplantation, can be a useful predictor of HCT outcomes.

Our analysis showed that leukocytosis in the first 3 months after HCT (WBC risk score 2) is a significant predictor of poor OS and PFS. In the case of high WBC, this may reflect the development of transplant-related complications such as infections, or presence of GVHD since WBC de-margination is expected from the high dose corticosteroids used as primary GVHD treatment. In the case of low WBC, this may reflect, marrow dysfunction associated with underlying malignancy, or defective marrow microenvironment. Leukopenia may also result from direct myelosuppression by infectious agents (especially viruses) or drugs. Finally, it is also possible that persistent low or high WBC early after HCT reflect impaired immunologic recovery, which predispose to infection or relapse.

We also proposed that the lower boundary for normal WBC for HCT prognostic purposes should be 2×10^9 cells/L, as opposed to the standard reference threshold of 4×10^9 cells/L. Indeed, our results suggest that patients with mild leukopenia (WBC 2-4 ×10^9 cells/L) after HCT have similar outcome as those with WBC 4-10 ×10^9 cells/L (HR=1.04 for OS, p=0.8).

We acknowledge that the single institution and retrospective design are inherent flaws of our study, although these limitations are mitigated to some degree by the large size of the cohort, the consistency of the results for RIC and MAC HCT, and the completeness of data at the different time points. As such, our results should be interpreted with some caution until it is validated in a large independent cohort. We also acknowledge that fluctuations of the WBC are expected in the course of routine clinical care, and thus the designation of prognostic value on single set point values in time needs to be interpreted cautiously, although this issue is not limited to this study only and is inherent to many studies with biologic measurements. It is reassuring, however, that our conclusions were also supported in analyses looking at the WBC trajectory over time, which is less likely to be affected by these fluctuations.

In summary, our finding suggest that the WBC at 1,2, and 3 months after HCT may have significant prognostic implications: patients with persistent leukopenia or leukocytosis in the first 3 months after HCT have severely compromised survival. Based on the WBC trajectory in the first 3 months after HCT, we have developed a WBC risk scoring system that effectively stratified 3 groups of patients with different PFS and OS. If validated, this system could potentially be a useful and readily available tool to guide prognostication and stratification of patients enrolled on HCT clinical trials, and alert clinicians toward earlier restaging of disease, closer monitoring of infection, donor chimerism, and potential pre-emptive interventions in patients with high risk scores.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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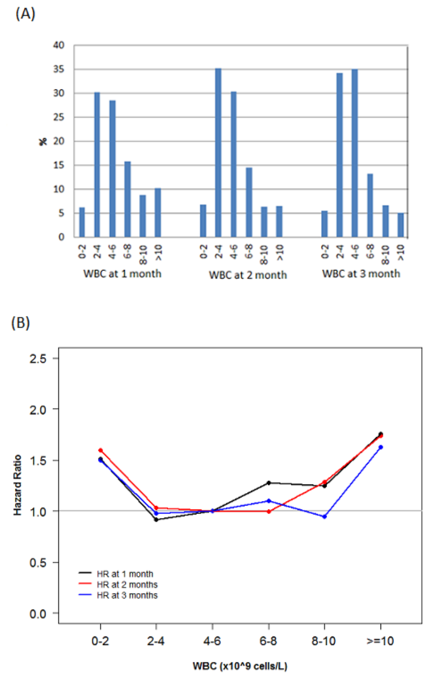

WBC ($x10^9$ cells/L)	0-2	2-4	4-6	6-8	8-10	>=10
HR at 1 month	1.51	0.91	1	1.28	1.25	1.76
p-value	0.0097	0.41	--	0.05	0.16	<.0001
HR at 2 months	1.60	1.03	1	1.00	1.29	1.74
p-value	0.0043	0.78	--	0.98	0.18	0.0021
HR at 3 months	1.50	0.98	1	1.10	0.94	1.62
p-value	0.0398	0.85	--	0.54	0.79	0.016

Figure 1.

(A) Distribution of WBC at 1,2,3 months after HCT. Landmark analysis was performed excluding relapse or death prior to each time point. (B) Hazard ratios of WBC at 1,2,3 months after HCT. The reference group is WBC 4.6 × 10^9 cells/L. The gray horizontal line represents hazard ratio of 1.

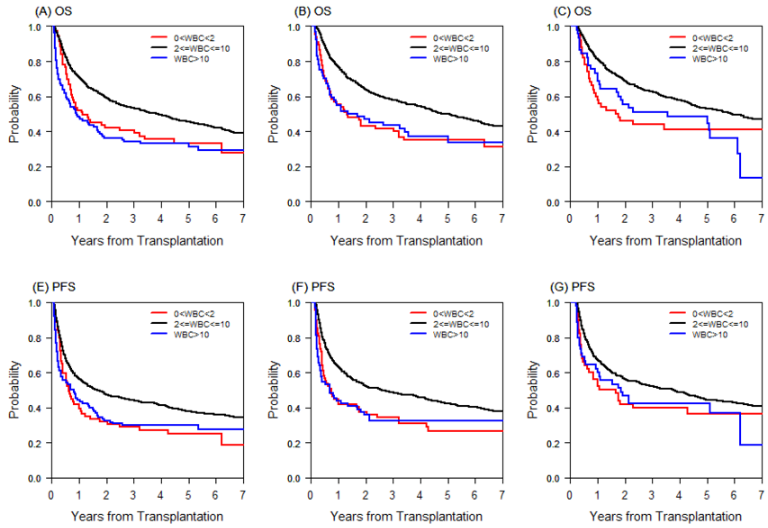

Figure 2.

(A) Overall survival (OS) by WBC category at 1 month after HCT. (B) OS by WBC category at 2 months after HCT. (C) OS by WBC category at 3 months after HCT. (D) Progression-free survival (PFS) by WBC category at 1 month after HCT. (E) PFS by WBC category at 2 months after HCT. (F) PFS by WBC category at 3 months after HCT.

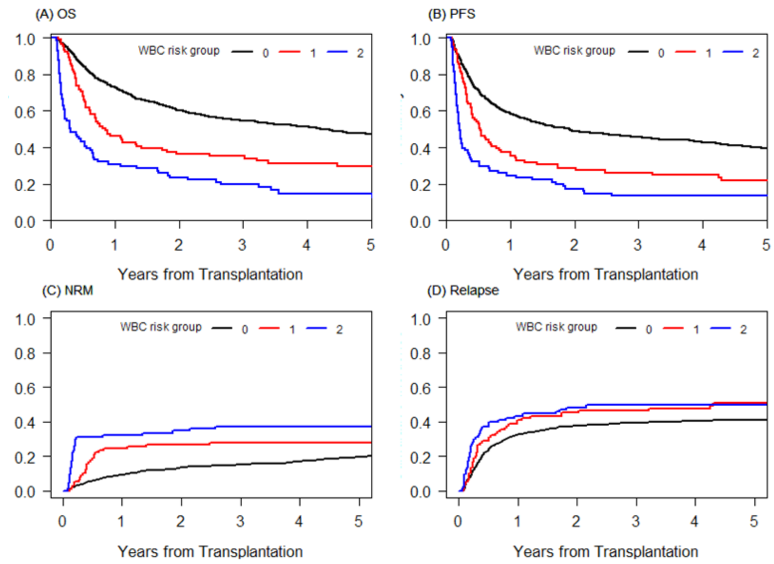

Figure 3.

(A) Overall survival, (B) progression-free survival, (C) cumulative incidence of NRM and (D) relapse by WBC risk group. 0: reference group, 1: low WBC during the first 3 months of HCT; 2: high WBC during the first 3 months of HCT.

Table 1

Baseline Characteristics

	All (N=1109)		MAC (N=528)		RIC (N=581)	
	N	%	N	%	N	%
Age, median (range)	51 (18, 74)		44 (18, 61)		57 (18, 74)	
Patient Sex						
Male	658	59.3	289	54.7	369	63.5
Female	451	40.7	239	45.3	212	36.5
Donor Sex						
Male	639	57.6	299	56.6	340	58.5
Female	470	42.4	229	43.4	241	41.5
Patinet-Donor Sex						
FF	205	18.5	111	21	94	16.2
FM	246	22.2	128	24.2	118	20.3
MF	265	23.9	118	22.3	147	25.3
MM	393	35.4	171	32.4	222	38.2
ECOG PS						
0	374	33.7	200	37.9	174	29.9
1	480	43.3	214	40.5	266	45.8
2-3	99	8.9	34	6.4	65	11.1
unknown	156	14.1	80	15.2	76	13.1
Disease						
AML	404	36.4	232	43.9	172	29.6
ALL	100	9	85	16.1	15	2.6
CLL	102	9.2	19	3.6	83	14.3
CML	78	7	61	11.6	17	2.9
Hodgkin lymphoma	50	4.5	8	1.5	42	7.2
Multiple Myeloma	35	3.2	1	0.2	34	5.9
MDS	130	11.7	50	9.5	80	13.8
Myeloproliperative Neoplasms	25	2.4	7	1.3	19	3.3
Non-Hodgkin lymphoma	180	16.2	62	11.7	118	20.3
Other Acute Leukemia	4	0.4	3	0.6	1	0.2
HLA Type						
Matched/Unrelated	565	50.9	245	46.4	320	55.1
Matched/Related	450	40.6	235	44.5	215	37
Mismatched/Unrelated	86	7.8	42	8	44	7.6
Mismatched/Related	8	0.7	6	1.1	2	0.3
Graft Source						
Bone Marrow	70	6.4	54	10.3	16	2.8
PBSC	1039	93.7	474	89.8	565	97.2
Disease Risk Index[a]						

	All (N=1109)		MAC (N=528)		RIC (N=581)	
	N	%	N	%	N	%
0: Low	177	16	57	10.8	120	20.7
1: Intermediate	591	53.3	278	52.7	313	53.9
2: High	304	27.4	171	32.4	133	22.9
3: Very high	37	3.3	22	4.2	15	2.6
Patient or Donor CMV seropositivity						
No	421	38	216	40.9	205	35.3
Yes	688	62	312	59.1	376	64.7
aGVHD prophylaxis						
CI+Sirolimus	697	62.8	288	54.5	409	70.4
CI+Other	376	33.9	229	43.4	147	25.3
Sirolimus+MMF	15	1.4			15	2.6
Ex vivo TCD/Other	21	1.9	11	2.1	10	1.7
Year of HCT, median (range)	2005 (2003, 2009)		2005 (2003, 2009)		2005 (2003, 2009)	
CD34 cells/kg ($\times 10^6$), medain (range)	7.8 (0.3, 47.7)		7.7 (0.3, 32.7)		7.9 (0.8, 47.7)	
no. of missing CD34	5	0.5	3	0.6	2	0.3
Day 30 Chimerism[b], median (range)					90 (0, 100)	
no. of missing chimerism					9	1.5
Years of follow-up for survivors median (range)	5.1 (1.1, 9.6)		5.9 (2.0, 9.2)		5.0 (1.1, 9.6)	

[a] Armand et al, Blood, 2012;

[b] patients with reduced intensity conditioning only. CI: Calcineurin Inhibitor; TCD: T cell depletion.

Table 2

WBC risk score assignment

WBC risk score	WBC 1m		WBC 2m		WBC 3m
0	Normal or High	&	Normal	&	Normal
	Normal	&	Normal		
	Normal	&			Normal
1	Low				
	Normal	&	Low		
2	High				
	Normal	&	High		

Blank means ‘any’ WBC category except that (Normal, Low, and Normal) or (High, Normal, and Normal) at 1,2,3 months after HCT, respectively, were categorized as WBC risk group 0. 4 patients with missing WBC at 2 months and 24 patients with missing WBC at 3 months after HCT were categorized based on 2 available WBC values. Scores for patients who died or relapsed after 1 month were based on their 1 month WBC values; scores for patients who died or relapsed after 2 months of HCT were based on their 1 and 2 months WBC values.

Table 3

Survival outcomes by WBC risk group

	WBC risk group[a]	Univariable Analysis			
		N	1-yr (%), (95% CI)	5-yr (%), (95% CI)	p-value[b]
OS	0	937	73 (70,76)	47 (44, 51)	ref
	1	91	46 (36, 56)	30 (21, 40)	<0.0001
	2	81	31 (21, 41)	15 (8, 24)	<0.0001
PFS	0	937	58 (55, 61)	39 (36, 43)	ref
	1	91	35 (26, 45)	22 (14, 31)	<0.0001
	2	81	25 (16, 34)	14 (7, 22)	<0.0001
NRM	0	937	9 (7, 11)	19 (17, 22)	ref
	1	91	24 (16, 33)	28 (19, 37)	0.008
	2	81	32 (22, 42)	37 (27, 48)	0.0005
Relapse	0	937	32 (29, 35)	41 (38, 44)	ref
	1	91	41 (30, 51)	51 (39, 61)	0.29
	2	81	43 (32, 54)	49 (38, 60)	0.0005

[a]0: referent group; 1: low WBC during the first 3 months of HCT; 2: high WBC during the first 3 months of HCT, 3: normal WBC initially but disease progression or relapse during the first 3 months of HCT.

[b]stratified by conditioning intensity

Table 4

Multivariable Cox models[a]

	OS				PFS				NRM				Relapse			
	HR	95% CI		p-value	HR	95% CI		p-value	HR	95% CI		p-value	HR	95% CI		p-value
Age[b]	1.27	1.08	1.49	0.005	1.11	0.95	1.30	0.19	1.34	1.02	1.76	0.036	0.99	0.82	1.20	0.93
Patient-Donor Sex																
M<-F vs. Other	1.08	0.90	1.30	0.39	1.03	0.86	1.23	0.76	1.10	0.82	1.49	0.53	1.00	0.81	1.25	0.98
HLA type																
MUD vs MRD	1.06	0.90	1.27	0.48	0.98	0.83	1.15	0.78	1.65	1.22	2.22	0.001	0.77	0.63	0.93	0.008
Mismatched vs MRD	1.26	0.94	1.68	0.12	1.08	0.82	1.43	0.59	1.95	1.25	3.03	0.003	0.80	0.55	1.15	0.23
Patient or Donor CMV																
seropositivity: Y vs. N	1.06	0.90	1.25	0.47	1.02	0.87	1.19	0.83	1.14	0.87	1.51	0.34	0.97	0.80	1.17	0.73
Disease Relapse Index																
Intermediate vs. Low	1.73	1.32	2.26	<.0001	1.93	1.50	2.49	<.0001	1.12	0.76	1.66	0.56	2.50	1.78	3.52	<.0001
High/Very high vs. Low	2.68	2.02	3.56	<.0001	2.98	2.28	3.89	<.0001	1.22	0.79	1.88	0.37	4.61	3.24	6.56	<.0001
Year of Transplantation	0.97	0.93	1.02	0.28	0.95	0.91	1.00	0.03	0.98	0.91	1.07	0.69	0.94	0.89	1.00	0.04
CD34 cells/kg ($\times 10^6$)[c]																
<=4 vs <4-15	1.28	0.99	1.66	0.06	1.31	1.03	1.66	0.03	1.37	0.90	2.09	0.15	1.21	0.91	1.63	0.19
>15 vs <4-15	1.20	0.94	1.53	0.15	1.17	0.93	1.49	0.19	0.83	0.53	1.32	0.43	1.33	1.01	1.76	0.045
ECOG performance Status																
1 vs. 0	1.31	1.07	1.58	0.007	1.29	1.07	1.54	0.007	1.21	0.89	1.66	0.23	1.32	1.05	1.65	0.017
2-3 vs. 0	1.58	1.18	2.11	0.002	1.42	1.08	1.88	0.01	1.89	1.19	3.00	0.007	1.26	0.89	1.79	0.20
missing vs. 0	1.29	0.99	1.67	0.06	1.23	0.96	1.58	0.10	1.08	0.69	1.67	0.75	1.31	0.96	1.78	0.09
Graft Source																
BM vs. PBSC	0.77	0.51	1.15	0.19	0.81	0.56	1.19	0.29	0.58	0.31	1.07	0.08	1.02	0.63	1.66	0.94
Grade 2-4 aGVHD	1.50	1.26	1.78	<.0001	1.19	1.00	1.42	0.05	2.28	1.75	2.98	<.0001	0.74	0.57	0.96	0.02
Gr. ≥3 Infection within D100	1.89	1.57	2.27	<.0001	1.71	1.40	2.08	<.0001	3.39	2.45	4.70	<.0001	1.14	0.88	1.48	0.33
WBC risk group																
1 vs. 0	1.64	1.26	2.13	0.0002	1.49	1.16	1.92	0.0017	2.04	1.34	3.11	0.0009	1.27	0.93	1.74	0.13
2 vs. 0	3.06	2.36	3.97	<.0001	2.88	2.23	3.73	<.0001	3.23	2.15	4.85	<.0001	2.86	2.03	4.03	<.0001

d: time dependent variable. MUD: matched unrelated donor; MRD: matched related donor.

[a] stratified by conditioning intensity.

[b] dichotomized as 1 if age>=40 in MAC or >=60 in RIC; 0 otherwise.

[c] 5 missing CD34 are combined with CD34<=4 because of a similar hazard ratio.