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Monocyte Subpopulation Recovery as Predictors of Hematopoietic Cell Transplantation Outcomes

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Monocyte recovery after hematopoietic cell transplantation (HCT) has been correlated with overall survival (OS). However, monocytes are heterogeneous and consist of classic ($CD14^{++}CD16^{-}$), intermediate ($CD14^{+}CD16^{+}$), and nonclassic ($CD14^{+}CD16^{++}$) subpopulations, with unique functional properties. We hypothesized that monocyte subpopulation reconstitution would vary based on allogeneic stem cell source and would be associated with outcomes. We studied monocyte subpopulation recovery at days 28, 60, 100, 180, and 365 post-HCT among 202 patients with hematologic malignancy. Significant differences in absolute monocyte count (AMC) and monocyte subpopulation counts at days 60 and 100 were identified based on stem cell source (all $P < .01$), with more robust recovery in umbilical cord blood (UCB) recipients. Using 2-fold cross-validation, optimal cutpoints were calculated for day 28 AMC and monocyte subpopulations based on OS. These were used to calculate hazard ratios for OS, disease-free survival (DFS), relapse, transplant-related mortality (TRM), and acute and chronic graft-versus-host disease. OS and DFS were superior when AMC and classic monocyte recovery were above optimal cutpoints (all $P < .03$). Relapse was reduced for those with AMC ($P < .01$) and classic ($P = .05$) monocyte counts above optimal cutpoints. TRM was also reduced when classic ($P = .02$) monocyte count exceeded optimal cutpoints. Intermediate and nonclassic monocyte recovery were not associated with outcomes. In summary, hematopoietic cell source is associated with monocyte subpopulation recovery, with the early robust recovery in UCB recipients. Recovery of AMC and classic monocytes were prognostic for survival, relapse, and TRM. These indicators may identify patients at increased risk for post-HCT failure and guide therapeutic interventions.

INTRODUCTION

Rapid monocyte recovery after hematopoietic cell transplantation (HCT) has been correlated with improved overall survival (OS) [1-4]. However, monocytes are heterogeneous and can be separated into 3 distinct phenotypic and functional subpopulations based on CD14 and CD16 surface expression: $CD14^{++}CD16^{-}$ (classic), $CD14^{+}CD16^{+}$ (intermediate), and $CD14^{+}CD16^{++}$ (nonclassic) monocytes [5,6]. In healthy individuals classic monocytes are most abundant in the peripheral blood (PB) and account for approximately 70% to 90% of the absolute monocyte count (AMC) [7,8]. Each subpopulation has unique functionality, although there are some inconsistencies across studies in the precise behavior of each subpopulation [9-17]. Classic monocytes have phagocytic activity, are less inflammatory, and are skewed toward the production of counter-regulatory cytokines, including IL-10 [10,15,18,19]. In contrast, nonclassic monocytes have a greater capacity to produce inflammatory cytokines on activation, including tumor necrosis factor-α and IL-1β [13], whereas intermediate monocytes are a transitional population, sharing features of both classic and nonclassic monocytes but with an inflammatory cytokine profile that is closer to nonclassic monocytes [13].

Monocyte subpopulations have been associated with unique states of health and disease. Classic monocytes have been described in the setting of tissue repair and innate immune functions [15,20], whereas nonclassic monocytes are important for immune patrolling and inflammatory functions.

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Both intermediate and nonclassic monocytes are expanded in states of acute inflammation, such as sepsis [11], or in chronic inflammation, such as systemic lupus erythematous [11] or obesity [9]. The recovery of monocyte subpopulations after HCT has not been thoroughly examined. A previous pilot study of a small population of pediatric and young adult patients (n = 30) undergoing HCT showed that the proportion of classic monocytes decreased during acute graft-versus-host disease (aGVHD), whereas the nonclassic and intermediate monocytes were increased. However, whether significant changes in absolute values of each of these populations occurred was not presented, and the small sample size limited the conclusions that could be drawn [21].

Monocytes isolated from umbilical cord blood (UCB) and adult blood have distinct transcriptional profiles and response to cytokine stimulation, correlating with differences between the functionality between these 2 cell sources [22]. To date it is unclear whether monocytes differ in their reconstitution based on the source of the HCT graft and whether this, in turn, is associated with transplant outcomes. Here we compare HCT graft source in terms of monocyte recovery and perform an analysis of monocyte subpopulation recovery and HCT outcomes.

METHODS

Patients included in this study underwent their first allogeneic HCT for any malignant diagnosis at the University of Minnesota between 2010 and 2014 and were enrolled onto an institutional immune reconstitution protocol (Figure 1). Demographic and clinical data were prospectively collected and managed in the University of Minnesota Bone Marrow Transplant Database. All participants and/or legal guardians provided informed consent according to the principles of the Declaration of Helsinki for inclusion in the immune reconstitution study and the University of Minnesota Bone Marrow Transplant Database before transplantation. The University of Minnesota Institutional Review Board approved the prospective collection of blood for immune reconstitution and clinical HCT data.

Pediatric and adult transplant recipients were included. Individuals who experienced grade II or greater aGVHD before day 28 were excluded because of the potential effects of GVHD treatment on monocyte recovery. Individuals with missing day 28 measurements, who relapsed, or who died before day 28 were excluded. AMC and absolute counts of classic, intermediate, and nonclassic monocyte subpopulations were assessed at days 28, 60, 100, and 365 after HCT.

Monocytes and subpopulations were identified based on their relative surface expression of CD14 and CD16. The gating strategy is shown in Supplemental Figure 1. Patient blood was processed in real time using ficolled samples. Cells were stained on the day they were received, fixed, and acquired by flow cytometry within 2 days of staining. At the time of each acquisition on the flow cytometer, an identically stained sample from third-party cryopreserved healthy donor cells was thawed, stained, and also acquired and analyzed to ensure that population frequencies remained constant, demonstrating antibody quality assurance over time.

Descriptive statistics for patient demographic and clinical data were performed for the overall sample and were based on stem cell source, including bone marrow (BM), PB, single UCB (SUCB), and double UCB (DUCB). Differences in the recovery of the AMC and absolute numbers of monocyte subpopulations (median and interquartile range) were evaluated between stem cell sources for each time point using nonparametric tests. To assess monocyte recovery and transplant outcomes, optimal cutpoints were calculated for the day 28 AMC and each monocyte subpopulation based on the Cox regression for the primary outcome, 2-year OS, adjusted for sex, conditioning intensity (myeloablative versus reduced intensity), and age group ($<$18, 18 to 44.9, and $\geq$45 years) for the entire sample. Adjusted variables were selected for inclusion in the regression models if significant ($P < .05$) in univariate analyses; variables tested included stem cell source, conditioning intensity, sex, GVHD prophylaxis regimen, recipient cytomegalovirus status, cytomegalovirus reactivation at day 100, disease risk, age group, and body mass index (BMI) group ($<$25 kg/m^2 or $\geq$25 kg/m^2).

For OS the inference for the binary group variable (less than optimal cutpoint versus at least an optimal cutpoint) was obtained by using the 2-fold cross-validation method [23] to avoid the inflated Type I error caused by the multiple tests when searching for the optimal cutpoint. Specifically, we randomly selected half of the sample as the training set to determine the cutpoint, which was associated with the highest significance of the binary variable in the multivariable Cox regression, to be used for defining the high/low groups for the other half of the sample; we then repeated this process by

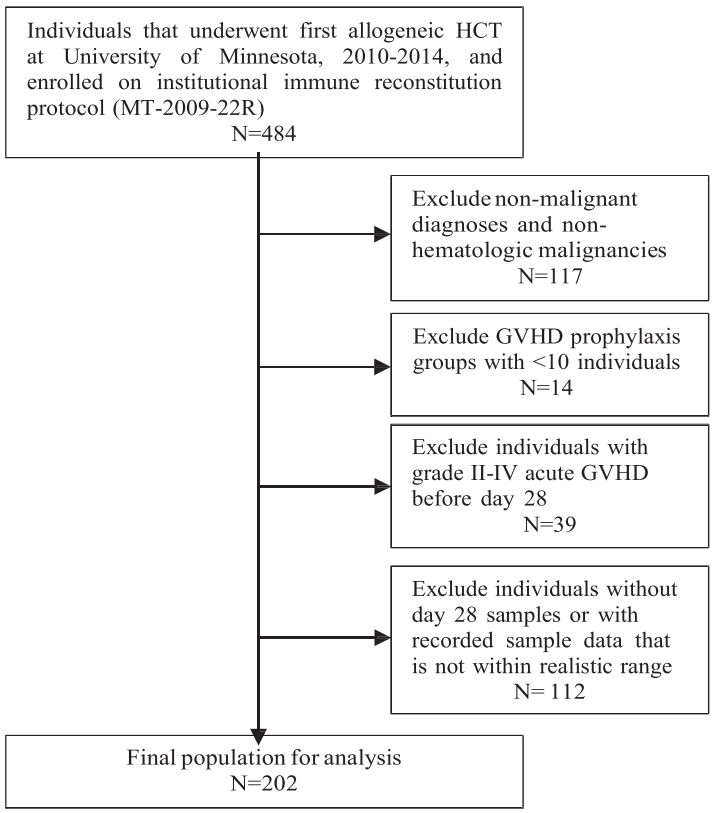

Figure 1. Study population. Summary of individuals included in the analysis, with inclusion and exclusion criteria.

switching the 2 subsets until the binary variable for the entire sample was determined. Finally, the hazard ratio (HR; for high versus low) and *P* value for the binary variable were calculated using the multivariable Cox regression stratified by the subset. The advantage of the 2-fold cross-validation method is to include all samples in both cutpoint searching and effect estimation without scarifying a Type I error. Cox regression or Fine-Gray regression was performed for other clinical endpoints, including the 1-year transplant-related mortality (TRM), 2-year disease-free survival (DFS), 2-year relapse, 100-day aGVHD, and 1-year chronic GVHD (cGVHD), using the same binary monocyte variables and covariates as for the OS.

All regression analyses started at day 28, the landmark time point. All tests were 2-sided, and $P < .05$ was considered statistically significant. Statistical analyses were performed in R 3.3.2 (R Core Team, Vienna, Austria, 2016).

RESULTS

Patient Characteristics

Among 202 participants, median age at the time of transplant was 50.9 years (range, 1.3 to 72.8), 36% were female, and 40% received myeloablative conditioning. Sixteen percent received BM grafts, 37% received PB, 10% received SUCB and 37% received DUCB. Additional patient, treatment, and disease characteristics are presented in Table 1.

Stem Cell Source and Monocyte Recovery

Monocyte subpopulation recovery was evaluated separately for BM, PB, SUCB, and DUCB at days 28, 60, 100, and 365 after HCT (Table 2, Figure 2). The recovery of the classic monocyte subpopulation was statistically significantly different between stem cell sources at day 28 ($P = .02$), with the most robust recovery seen in SUCB recipients, but AMC and other subpopulations did not differ based on stem cell source (all $P > .05$). By day 60, along with significant differences in the recovery of the classic monocyte subpopulation ($P < .01$), there were also differences in the recovery of the intermediate monocyte population ($P < .01$) and AMC ($P < .01$), with SUCB and DUCB recipients showing significantly greater absolute numbers compared with BM or PB stem cells (PBSCs). These differences persisted at day 100 ($P < .01$ for AMC, classic, and intermediate monocytes), although AMC and subpopulation values were higher at day 60 compared with day 100. By day 365 only intermediate monocytes remained significantly different across stem cell sources, with SUCB recipients at least 2.5-fold greater than BM, PBSC, or DUCB recipients ($P < .01$).

Monocyte Recovery and Clinical Outcomes

Using day 28 post-HCT values, the optimal cutpoint for AMC was $.09 \times 10^9$/L, for nonclassic monocytes $.02 \times 10^9$/L, for intermediate monocytes $.03 \times 10^9$/L, and for classic monocytes $.07 \times 10^9$/L. The distribution of individuals falling above and below the cutpoints is shown in Table 3. In multivariable

Table 1
Patient and Treatment Characteristics

	All Groups (N = 202)	BM (n = 32)	PBSC (n = 75)	SUCB (n = 21)	DUCB (n = 74)	*P*
Patient sex	73 (36.1)	14 (43.8)	25 (33.3)	8 (38.1)	26 (35.1)	.77
Female						
Median age at HCT, yr (range)	50.9 (1.3-72.8)	36.1 (1.6-69.4)	57.0 (21.2-72.8)	9.4 (2.3-70.7)	53.6 (1.3-71.9)	<.01*
Age group						<.01*
0-18 yr	40 (19.8)	11 (34.4)	0	18 (85.7)	11 (14.9)	
18-45 yr	41 (20.3)	7 (21.9)	16 (21.3)	1 (4.8)	17 (23.0)	
≥45 yr	121 (59.9)	14 (43.8)	59 (78.7)	2 (9.5)	46 (62.2)	
Median follow-up, days (range)	741.0 (33.0-2229.0)	737.5 (39.0-2159.0)	736.0 (33.0-1974.0)	728.0 (37.0-2229.0)	762.5 (34.0-1967.0)	.81
Disease						<.01*
ALL	46 (22.8)	7 (21.9)	9 (12.0)	15 (71.4)	15 (20.3)	
AML	66 (32.7)	5 (15.6)	29 (38.7)	0	32 (43.2)	
CML	10 (5.0)	8 (25.0)	1 (1.3)	0	1 (1.4)	
Other leukemia	10 (5.0)	1 (3.1)	4 (5.3)	0	5 (6.8)	
Myelodysplasia	32 (15.8)	2 (6.3)	15 (20.0)	3 (14.3)	12 (16.2)	
Non-Hodgkin lymphoma	17 (8.4	3 (9.4)	8 (10.7)	3 (14.3)	3 (4.1)	
Hodgkin lymphoma	10 (5.0)	2 (6.3)	5 (6.7)	0	3 (4.1)	
Myeloproliferative disease	1 (.5)	1 (3.1)	0	0	0	
Multiple myeloma	10 (5.0)	3 (9.4)	4 (5.3)	0	3 (4.1)	
Disease risk group						.37
Standard	109 (54.0%)	14 (43.8%)	38 (50.7%)	12 (57.1%)	45 (60.8%)	
High	93 (46.0%)	18 (56.3%)	37 (49.3%)	9 (42.9%)	29 (39.2%)	
Median time from diagnosis to HCT, mo (range)	6.5 (2.0-376.7)	13.2 (3.5-76.6)	6.1 (2.0-125.9)	25.1 (3.0-76.3)	4.9 (2.3-376.7)	.02*
Recipient CMV status						.22
Positive	105 (52.0)	16 (50.0)	33 (44.0)	13 (61.9)	43 (58.1)	
Negative	96 (47.5)	16 (50.0)	42 (56.0)	7 (33.3)	31 (41.9)	
Missing	1 (.5)	0	0	1 (4.8)	0	
Conditioning intensity						<.01*
Myeloablative	81 (40.1)	16 (50.0)	20 (26.7)	19 (90.5)	26 (35.1)	
Reduced intensity	121 (59.9)	16 (50.0)	55 (73.3)	2 (9.5)	48 (64.9)	
GVHD prophylaxis						<.01*
CSA/MMF	112 (55.4)	9 (28.1)	38 (50.7)	17 (81.0)	48 (64.9)	
CSA/MMF+ATG	32 (15.8)	7 (21.9)	14 (18.7)	3 (14.3)	8 (10.8)	
CSA/MTX	41 (20.3)	16 (50.0)	23 (30.7)	1 (4.8)	1 (1.4)	
SIRO/MMF	17 (8.4)	0	0	0	17 (23.0)	
ATG, yes	32 (15.8)	7 (21.9)	14 (18.7)	3 (14.3)	8 (10.8)	.43

Values are n (%) unless otherwise defined. ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, antithymocyte globulin; CML, chronic myeloid leukemia; CMV, cytomegalovirus; CSA, cyclosporine; MMF, mycophenolate mofetil; MTX, methotrexate; SIRO, sirolimus.

* $P < .05$.

Table 2
Monocyte Subpopulation Recovery ($\times 10^9$/L) Post-HCT by Stem Cell Source

	Monocyte Type	BM (n = 32)	PBSC (n = 75)	SUCB (n = 20)	DUCB (n = 74)	*P*
Day 28	AMC	.63 (.41-.96)	.53 (.33-.86)	.66 (.48-1.12)	.51 (.27-.83)	.10
	Nonclassic	.03 (.02-.05)	.02 (.01-.04)	.04 (.02-.08)	.03 (.01-.07)	.08
	Intermediate	.08 (.04-.14)	.06 (.04-.09)	.08 (.06-.11)	.08 (.04-.16)	.20
	Classic	.47 (.24-.69)	.38 (.23-.60)	.48 (.32-.75)	.27 (.16-.53)	.02*
		(n = 20)	(n = 59)	(n = 8)	(n = 42)	
Day 60	AMC	.40 (.18-.68)	.45 (.24-.62)	1.27 (.59-1.46)	.95 (.55-1.27)	<.01*
	Nonclassic	.02 (.01-.04)	.03 (.01-.04)	.04 (.01-.08)	.04 (.02-.08)	.05
	Intermediate	.03 (.02-.06)	.04 (.03-.06)	.06 (.05-.19)	.08 (.05-.17)	<.01*
	Classic	.30 (.11-.49)	.31 (.16-.45)	.92 (.48-1.22)	.70 (.43-.96)	<.01*
		(n = 17)	(n = 45)	(n = 9)	(n = 37)	
Day 100	AMC	.44 (.20-.53)	.26 (.17-.38)	.9 (.68-.95)	.52 (.34-.80)	<.01*
	Nonclassic	.01 (.0-.05)	.02 (.01-.03)	.03 (.01-.03)	.02 (.01-.05)	.23
	Intermediate	.04 (.02-.06)	.03 (.02-.06)	.06 (.05-.09)	.07 (.04-.10)	<.01*
	Classic	.33 (.16-.38)	.19 (.10-.29)	.69 (.58-.78)	.40 (.23-.70)	<.01*
		(n = 16)	(n = 27)	(n = 6)	(n = 32)	
Day 365	AMC	.43 (.30-.65)	.48 (.29-.68)	.39 (.24-.55)	.51 (.33-.83)	.58
	Nonclassic	.01 (.01-.03)	.02 (.01-.04)	.02 (.01-.03)	.03 (.01-.04)	.24
	Intermediate	.03 (.02-.04)	.03 (.02-.08)	.09 (.06-.13)	.06 (.04-.09)	<.01*
	Classic	.33 (.21-.52)	.35 (.19-.46)	.30 (.17-.41)	.41 (.23-.64)	.54

Values are median (interquartile range).
* $P < .05$.

regression analyses, adjusted for sex, conditioning intensity, and age at transplant, multiple significant associations were identified between monocyte recovery and post-HCT outcomes. For 2-year OS, after 2-fold cross-validation, high day 28 AMC (HR, .44; 95% confidence interval [CI], .21 to .92; $P = .03$) and high classic monocyte count (HR, .30; 95% CI, .14 to .65; $P < .01$) were significantly associated with decreased risk of death (Table 3, Figure 3). Similarly, for 2-year DFS, AMC (HR, .30; 95% CI, .13 to .69; $P < .01$) and classic monocyte (HR, .29; 95% CI, .15 to .56; $P < .01$) recovery above the optimal cutpoint at day 28 were associated with superior DFS (Figure 3). Intermediate and nonclassic monocyte counts were not associated with OS or DFS. Risk of relapse at 2 years was less for individuals with AMC (HR, .29; 95% CI, .12 to .74; $P < .01$) and classic monocyte (HR, .46; 95% CI, .21 to 1.00; $P = .05$) recovery above specified optimal cutpoints at day 28 (Figure 3). Nonclassic and intermediate monocyte recovery were not associated with relapse. One-year TRM was associated with classic monocyte subpopulation recovery (HR, .24; 95% CI, .07 to .80; $P = .02$) (Figure 3) but not with AMC or the recovery of other monocyte subpopulations. Day 100 grades II to IV and III to IV aGVHD and 2-year cGVHD were not associated with day 28 AMC or subpopulation recovery in adjusted models.

DISCUSSION

Prior studies demonstrate that absolute monocyte recovery is associated with transplant outcomes [1-4]. However, monocytes are heterogeneous, and to date no study has investigated the recovery of monocyte subpopulations and determined whether they are associated with HCT outcomes. Here we used a standard approach of classifying monocyte subpopulations based on CD14 and CD16 expression to understand whether reconstitution of these subpopulations varies based on stem cell source and whether the kinetics of recovery were associated with transplant outcomes. Stem cell source was associated with monocyte subpopulation recovery; however, in univariate analyses stem cell source was not significantly associated with clinical outcomes and was not included in multivariate models. Through the use of 2-fold cross-validation, optimal cutpoints were identified for AMC and monocyte subpopulations, and recipients with cell counts above those cutpoints were found to have improved survival and decreased relapse and TRM.

There was considerable variability in AMC and monocyte subpopulation recovery within each stem cell source. Differences appeared to be most clinically relevant, in terms of absolute differences, at days 60 and 100. Interestingly, individuals who received UCB transplantation showed more robust recovery of the total monocyte population and for all subpopulations at days 60 and 100. However, these differences between UCB and other stem cell sources were mostly resolved by day 365, and differences in HCT outcomes were not observed based on stem cell source within univariate analyses. It is possible that higher numbers of monocytes after UCB is due to delayed T cell recovery and proportionately higher monocytes or due to inherent differences in the hematopoietic stem cells present in the fetal sources (ie, UCB) compared with adult stem cell sources (ie, PBSCs or BM). The AMC and monocyte subpopulation absolute values peaked at day 60 and remained relatively stable thereafter. Interestingly, the recipients of SUCB and DUCB transplantation showed more robust monocyte recovery relative to other stem cell sources, despite having distinct clinical demographic characteristics, notably recipient age at the time of HCT and underlying disease, suggesting that host factors are perhaps less important than stem cell source for monocyte recovery.

For the entire cohort we observed significantly different transplant outcomes based on AMC and classic monocyte subpopulation recovery. Patients with recovery of these populations below the optimal cutpoints experienced inferior OS and DFS; furthermore, those with AMC and classic monocyte recovery below the optimal cutpoint also showed increased relapse, and those with classic monocyte recovery below the optimal cutpoint experienced increased TRM. These findings are consistent with observations made in previous studies [1-4], although the calculated AMC cutpoint in the present analysis is lower than what has been used in previous analyses based on differences in populations and methodology.

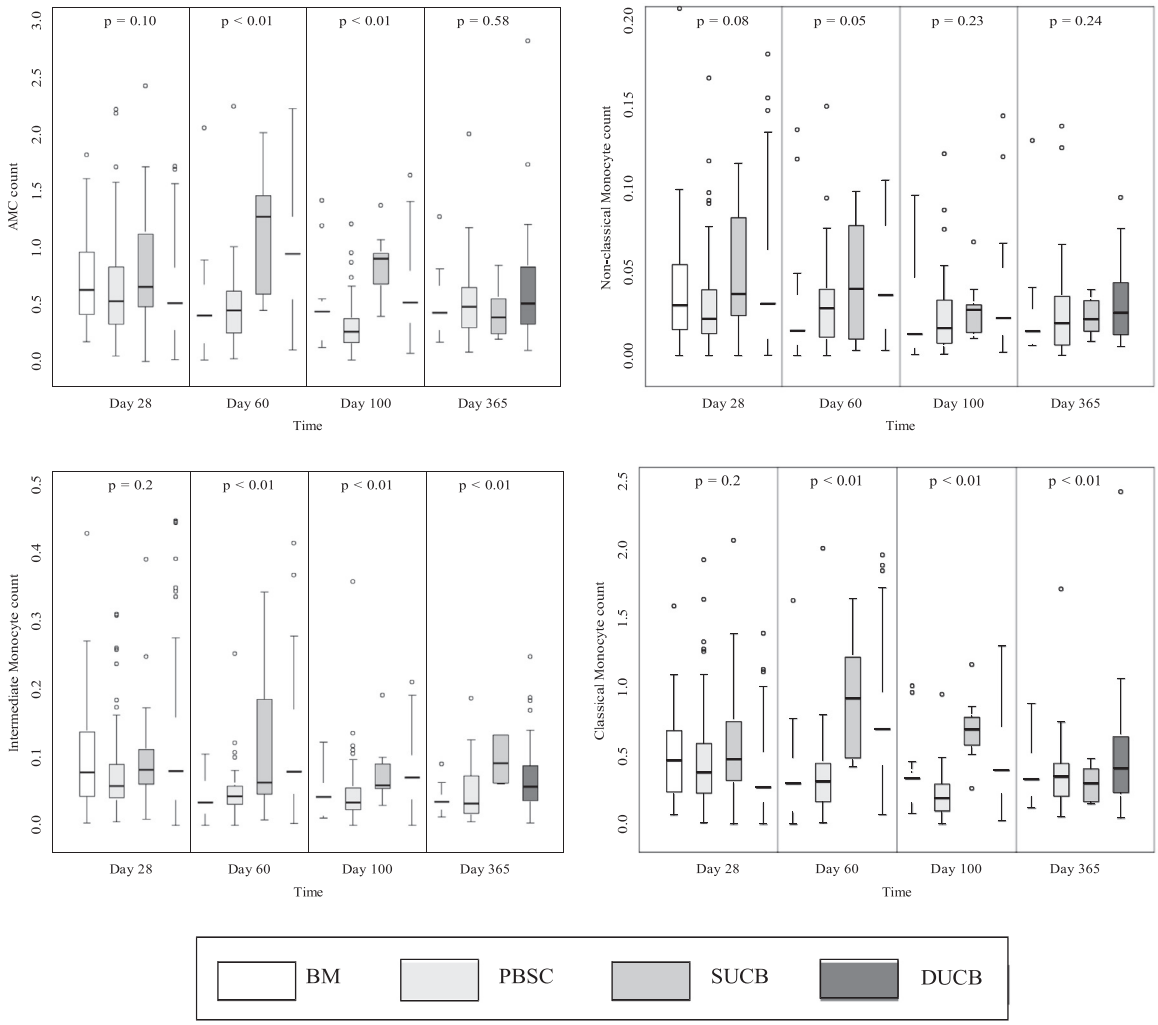

Figure 2. Monocyte subpopulation recovery ($\times 10^9$/L) post-HCT, by stem cell source and day. Interquartile ranges are shown with the bottom and top indicating the 25th and 75th percentiles, the thick line indicating the median, whiskers representing 1.5 times of the height of the box (or minimum/maximum values if there is no value in that range), and the circle indicating the outliers.

It is somewhat anticipated that individuals with more robust BM recovery would have superior outcomes, but we did not see this for all outcomes within the analysis. This supports the assertion that these cells play important roles in immune recovery, perhaps through their cytokine production or antigen presentation, which may lead to protection from relapse. Interestingly, nonclassic monocyte recovery, which would be expected to enhance inflammatory capacity and immune patrolling, was not associated with any post-HCT outcomes. Given the increased systemic inflammation expected with post-HCT conditions like sepsis, GVHD, or veno-occlusive disease, which may lead to subsequent mortality, we had hypothesized potential associations between nonclassic monocyte recovery and endpoints such as TRM, DFS, or OS; however, this was not observed. One potential explanation for this finding is that that this study used circulating PB monocyte analysis, and changes in the frequency of monocyte populations associated with GVHD may be occurring at the tissue level and not in the PB.

The lack of differences in the incidence of aGVHD between the groups may be multifactorial. The most feasible explanation is that individuals who developed grades III to IV aGVHD before day 28 (n = 19) were excluded from the analysis because it was believed their inclusion and the effect of GVHD therapies on monocyte recovery would alter results. Although these same concerns can be raised for patients who developed grades III to IV aGVHD after this time point, individuals fitting this description were relatively minimal (n = 30). The lack of association between AMC or monocyte subpopulation

Table 3
OS Analysis of Individuals Falling Above and Below the Optimal Cutpoints of the Monocyte Subpopulation

Monocyte Population	Optimal Cutpoint($\times 10^9$/L)	N (<: ≥ cutpoint)	Estimated HR for High vs. Low (95% CI)	*P*
AMC	.09	10:188	.44 (.21-.92)	.03*
Nonclassic	.02	73:125	.93 (.56-1.54)	.77
Intermediate	.03	27:171	.60 (.33-1.10)	.10
Classic	.07	17:181	.30 (.14-.65)	<.01*

* $P < .05$

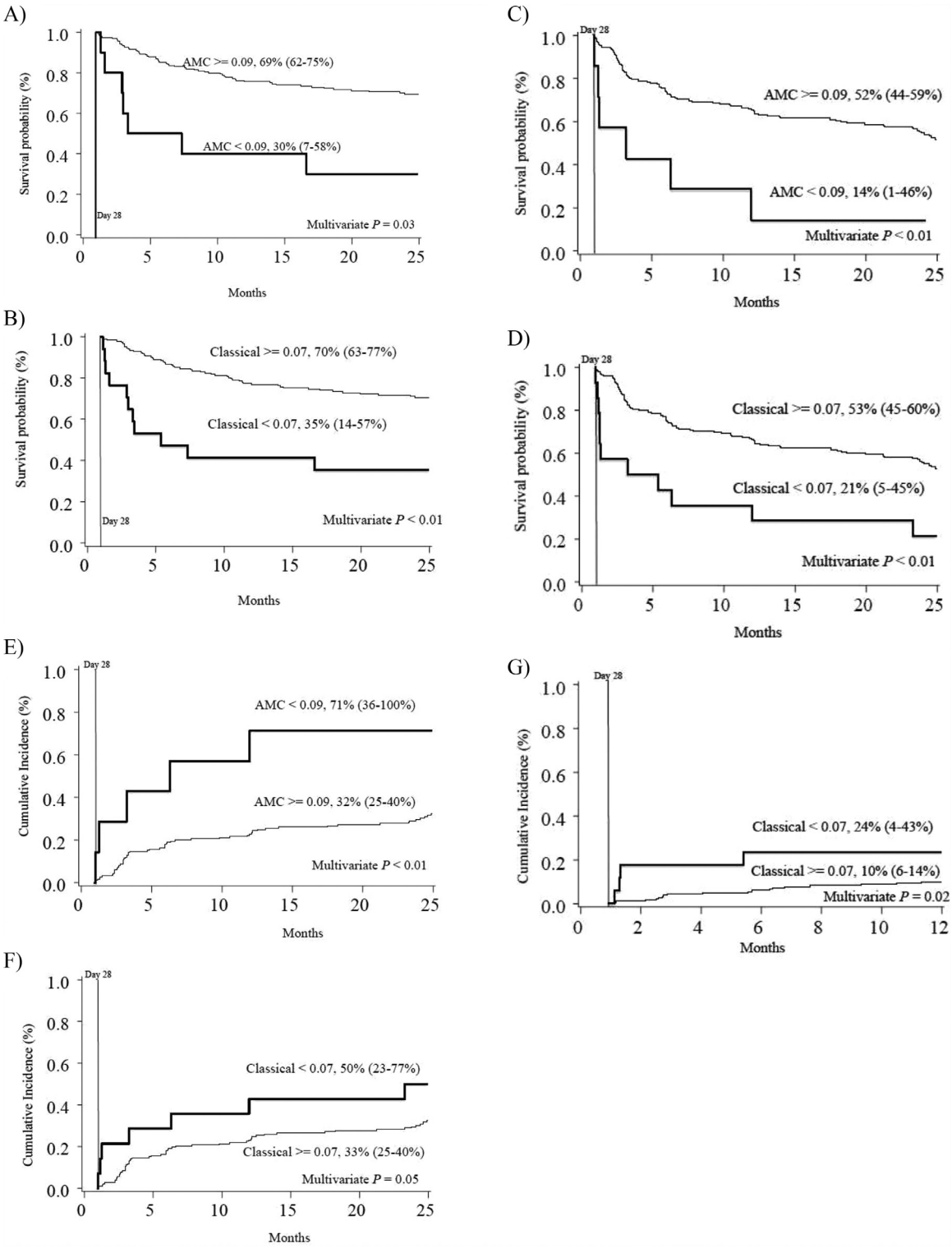

Figure 3. Two-year OS (A and B),[1] DFS (C and D),[1] relapse (E and F),[2] and 1-year TRM (G)[2] of individuals above and below the optimal cutpoints ($\times 10^9$/L), based on AMC, and classic monocyte count. The optimal cutpoints were selected based on the whole sample. A-C, Kaplan-Meier estimates; E-G, cumulative incidence estimates. *P* values were calculated from the 2-fold cross-validation method based on the multivariable regression adjusting for sex, conditioning intensity, and age at transplant.

recovery and cGVHD may in part be related to the exclusion of individuals who developed early aGVHD (ie, before day 28). In fact, Moon et al. [24] compared patients who developed aGVHD before and after day 28 and found significantly more cGVHD in the former group. It is also possible that the latency between day 28 monocyte recovery and the development of cGVHD is too distant to see a meaningful relationship or that there is no relationship.

Although we did not have data on other sources of inflammation, such as lifestyle factors or underlying health conditions such as autoimmune disease or type 2 diabetes, we did have the BMI at the time of transplant. Chronic inflammation is well described in the setting of overweight and obesity [25-27]. We hypothesized that individuals with increased BMI may have more inflammatory monocyte subpopulation recovery compared with those who are nonoverweight/nonobese (BMI $< 25 kg/m^2$) and possibly inferior clinical outcomes; however, we did not see significant associations between BMI group and clinical outcomes in univariate analyses and did not see associations between monocyte subpopulation recovery and BMI group (data not shown), even when the analysis was restricted to adults only, possibly consistent with the reports suggesting no impact of recipient obesity on transplant outcomes [28-30].

This analysis provides valuable data regarding the prognostic role of monocyte subpopulation recovery after HCT; however, there are limitations to this study. We do not report concurrent results of other WBC lineages, which may be similarly predictive of HCT outcomes. This approach was taken because the inclusion of additional WBC lineages would have diminished our power to draw conclusions on our primary hypotheses regarding the importance of monocyte subpopulation recovery. In addition, the monocyte counts were obtained at predetermined time points and may not be reflective of other time points or clinical events in the post-HCT course, thus not fully capturing the monocyte-associated predictive value or response to transplant-related health effects. Furthermore, we do not have functional data to interrogate the behavior of these monocyte subpopulations at the defined post-HCT time points. Although we observed numerical differences in UCB and adult stem cell source monocyte recovery, it has previously been demonstrated that UCB monocytes are also functionally unique; they respond differently to cytokine stimulation and exhibit increased sensitivity to activation of key signaling pathways [22]. Additionally, previous work has shown that among PBSC recipients, despite numerical monocyte reconstitution early in the post-HCT course, functional recovery, as evidenced by oxidative burst after stimulation with phorbol 12-myristate 13-acetate (PMA), was impaired until day 90 post-HCT [31]. Thus, a potential future direction for study is to understand whether the monocyte phenotype and function are similar after engraftment and beyond across donor sources and at what point function is appreciably recovered. Of note, the methodology adopted in this study for identifying cutpoints for a continuous monocyte recovery marker focused on the marker itself while adjusting for important patient and treatment characteristics. However, if the overall predictive power of the combined covariates including the marker were of interest, the survival receiver operating curve method could be used [32]. Despite the stated limitations, this study presents a single-institution HCT population with standardized supportive care guidelines, GVHD treatment approaches, and relatively homogenous conditioning regimens that can be associated with comprehensive monocyte subpopulation immune reconstitution data [33], which is a notable strength and sets this study apart from others that have reported on AMC and HCT outcomes.

Herein, we have shown that hematopoietic cell source is associated with monocyte subpopulation recovery after HCT in that recipients of UCB demonstrated more robust monocyte recovery in the first 100 days post-HCT compared with BM and PBSC recipients. We have also shown that optimal cutpoints based on early (day 28) monocyte subpopulation recovery after HCT are prognostic using the cross-validation method. If further validated, these simple and easily accessible indicators could be used prospectively to identify patients at increased risk for adverse outcomes and may direct supportive care efforts. Additional functional analyses of monocyte subpopulations could further define the etiology of the demonstrated outcome advantages.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.bbmt.2019.01.003.

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