Biol Blood Marrow Transplant 25 (2019) 12–18

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Proinflammatory Cytokine and Adipokine Levels in Adult Unrelated Marrow Donors Are Not Associated with Hematopoietic Cell Transplantation Outcomes

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Article history:
Received 23 April 2018
Accepted 10 August 2018

Key Words:
Cytokines
GVHD
HCT donor

A B S T R A C T

Graft-versus-host disease (GVHD) is a frequent cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (allo-HCT). GVHD occurs when donor lymphocytes are activated by inflammatory cytokines and alloantigens. The role of donor biologic characteristics, such as basal inflammation, has not been investigated as a risk factor for GVHD but is theoretically transferrable to the recipient. We evaluated donor serum and plasma concentrations of cytokines and adipokines (IL-1β, IL-6, tumor necrosis factor [TNF]-α, leptin, suppression of tumorigenicity-2, and adiponectin) from test (n = 210) and replication (n = 250) cohorts of matched, unrelated transplant peripheral blood stem cell recipients identified through the Center for International Blood and Marrow Transplantation Research between 2000 and 2011 for hematologic malignancies. Hazard ratios were estimated for acute (grades II to IV and III to IV) and chronic GVHD, overall survival, disease-free survival, transplant-related mortality, and relapse for each cytokine or adipokine, adjusting for significant covariates. The lowest cytokine quartile was considered as the reference group for each model. To account for multiple testing $P < .01$ was considered the threshold for significance. In the test cohort a borderline significant association was identified between donor serum IL-1β concentrations and grades III to IV acute GVHD in the recipient ($P = .01$), and a significant inverse association was identified between donor TNF-α concentrations and chronic GVHD ($P = .006$). These findings were not validated in the replication cohort. Although the initial associations between cytokine levels and allo-HCT outcomes were not validated, the idea that donor characteristics may be transferable to the recipient remains an exciting area for future research.

INTRODUCTION

Use of allogeneic hematopoietic cell transplantation (allo-HCT) has increased dramatically over the last 3 decades and is now considered a potentially curative therapy for multiple malignant and nonmalignant diseases [1]. Despite the potential for long-term cure, allo-HCT is associated with significant risks for morbidity and mortality. One of the most frequent complications is graft-versus-host disease (GVHD), which is driven by a cascade of conditioning-induced tissue injury, proinflammatory cytokines, and activation of donor immune cells that recognize host tissue alloantigens, all culminating in tissue destruction by donor T cells [2,3]. GVHD affects nearly one-half of HCT recipients [4,5]. Efforts to decrease risk for GVHD through improved donor selection algorithms and refinement of GVHD prophylactic strategies have been made [5]; however, the incidence of GVHD remains high. Although

Financial disclosure: See Acknowledgments on page 17.
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https://doi.org/10.1016/j.bbmt.2018.08.011
1083-8791/

considerable effort has focused on donor–recipient matching, given that identically HLA-matched donors differ in their rates of GVHD, the capacity to induce GVHD between donors and recipients varies for unknown reasons. To date, biologic factors of HCT donors have not been explored in detail and represent a potential avenue for further progress in improving HCT outcomes.

We hypothesized that donor biologic characteristics, such as their basal systemic inflammatory capacity, may be transferrable to HCT recipients. This hypothesis was based on murine models in which proinflammatory hematopoietic cells isolated from obese mice were serially transplanted into normal-weight recipient mice. These stem cells gave rise to higher numbers of myeloid progenitors with increased inflammatory capacity [6]. Prior studies show that the proinflammatory cytokines tumor necrosis factor (TNF)-α and IL-1β play key roles in GVHD initiation in murine models [2,7]. Additional biomarkers, such as suppression of tumorigenicity-2 (ST2), IL-6, leptin, and adiponectin, have been shown to regulate systemic inflammation, and recipient serum/plasma concentrations have been associated with the risk of acute and chronic GVHD [8-11]. Given that inflammation may be transferrable from donor to recipient and that inflammatory cytokines are critical in the induction and propagation of GVHD, we hypothesized that HCT donors with more systemic inflammation, regardless of the underlying cause, will increase the risk for subsequent GVHD and other adverse outcomes in unrelated donor allo-HCT recipients. We investigated this relationship using a cohort of paired HCT donors and recipients from the Center for International Blood and Marrow Transplantation Research (CIBMTR) where serum or plasma samples and mature clinical follow-up were available.

METHODS

Data Source

Data were obtained from the CIBMTR, which is a collaboration between the Medical College of Wisconsin and the National Marrow Donor Program/Be the Match. Data from consecutive autologous and allogeneic HCTs are collected longitudinally from more than 500 transplant centers worldwide. The quality and compliance of data submission is monitored through computerized data checks, physician reviews, and regularly scheduled data audits. CIBMTR observational studies are conducted under informed consent from the included donors and patients in compliance with all human subject protections and regulations and approved by the National Marrow Donor Program Institutional Review Board.

Subjects

Individuals included in the CIBMTR patient registry with a primary diagnosis of acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myeloid leukemia, or myelodysplastic syndrome (MDS), who underwent their first allogeneic HLA-A, -B, -C and -DRB1 matched (8/8 matched) unrelated peripheral blood stem cell (PBSC) transplant between 2000 and 2011 were included in the study. Individuals who received ex vivo T cell–depleted or $CD34^+$ selected grafts, transplants from multiple donors, or who had missing donor data were excluded from the analysis. Individuals who did not have pre-HCT donor samples available for cytokine analysis were also excluded.

A test cohort and a replication cohort were used to validate significant associations. The test cohort sample size was determined based on availability of donor serum samples for a subset of recipients who underwent HCT between 2000 and 2005. The 210 samples used for the test cohort represent all available serum samples, based on eligibility criteria and the defined time period. Based on the significant associations from the test cohort, power calculations were performed to determine the necessary sample size to validate those results in the replication cohort. Using 80% power, α value of .05, and the differences detected in the test cohort, we calculated a sample size of approximately 250 to detect the same differences for outcomes of interest. Additional samples and additional serum samples from the time frame of the initial cohort were not available. For the replication cohort 579 donor plasma samples were available between 2004 and 2011; 250 samples were selected, matched to the test cohort as closely as possible based on known GVHD risk factors, including recipient age and conditioning regimen intensity.

Cytokine Analyses

Donor samples were collected before granulocyte colony-stimulating factor (G-CSF) administration. Serum samples from clot activator tubes were used from the test cohort, and plasma samples derived from ACD-A tubes were used from the replication cohort. All samples were cryopreserved. Previous paired serum and plasma samples have yielded stable and reproducible cytokine results [12]. Cytokine analyses were performed on samples using the Luminex platform (R&D Systems, Minneapolis, MN). All testing was performed in the University of Minnesota Cytokine Reference Laboratory, which is a Clinical Laboratory Improvement Amendments–certified laboratory. Cytokines tested were IL-6, IL-1β, TNF-α, ST2, leptin, and adiponectin. All samples were tested in duplicate, and the values used for each patient are the means of the replicates. Cytokines were discretized into quartiles for analysis.

Statistical Methods

Descriptive statistics were performed. Primary endpoints included incidence of grades II to IV and grades III to IV acute GVHD and chronic GVHD. Acute GVHD grades II to IV was graded according to consensus criteria at day 100 with death without acute GVHD as a competing risk. Chronic GVHD was reported as cumulative incidence at 6 months, 1 and 2 years after HCT with death without chronic GVHD as a competing risk. Secondary outcomes included relapse, disease-free survival (DFS), nonrelapse mortality, and overall survival (OS). Relapse was reported as a cumulative incidence with nonrelapse mortality as a competing risk. DFS was defined as time to treatment failure either death or relapse. Nonrelapse mortality was defined as death in continuous remission with relapse as a competing risk.

Patient-, disease-, and transplant-related variables considered in analyses included recipient and donor age at HCT, recipient and donor race, donor–recipient sex match, donor and recipient cytomegalovirus status, recipient Karnofsky score before HCT, disease status at HCT, interval from diagnosis to HCT, HCT conditioning intensity [13], use of total body irradiation in conditioning, $CD34^+$ cell dose, GVHD prophylaxis, and use of antithymocyte globulin (ATG) or alemtuzumab.

Hazard ratios were estimated for acute (grades II to IV and III to IV) and chronic GVHD, OS, DFS, transplant-related mortality (TRM), and relapse for each cytokine or adipokine. The lowest cytokine quartile was considered the reference group for each model. Cox's proportional hazards models [14] were used to adjust for significant covariates. A stepwise forward model selection was used to identify significant covariates to be included in the models with a threshold of $P < .05$ for variable entry and exit. Transplant center was adjusted as a random effect in all models. To account for multiple comparisons, $P < .01$ was considered significant.

RESULTS

Patient and donor characteristics are shown in Table 1. Among the 210 patients included in the test cohort 47% were female, the median age at transplant was 45 years (range, 6 to 75), and 63 transplant centers are represented, with transplants occurring between 2000 and 2005. The most common diagnosis was AML (46%), followed by MDS (20%), ALL (18%), and chronic myeloid leukemia (17%). Seventy percent of transplants used myeloablative conditioning. Median donor age was 36 years (range, 20 to 60), and 47% of donors were female. For the 250 patients included in the replication cohort 48% were female, the median age at transplant was 48 years (range, 6 to 70), and 71 transplant centers were represented, with transplants occurring between 2005 and 2011, all but 2 between 2008 and 2011. The most common diagnosis was ALL (72%), followed by MDS (18%) and AML (9%). Conditioning intensity was most frequently myeloablative (79%). Median donor age was 30 years (range, 19 to 58), and 30% of donors were female.

Significant differences between the test and replication cohorts are noted in Table 1. Notably, in the test cohort donor sex was more frequently female ($P < .001$), and total body irradiation was more frequently used ($P < .001$). Conversely, ATG was used more frequently used in the replication cohort ($P < .001$). Regarding GVHD prophylaxis, it was more likely to be cyclosporine and methotrexate in the test cohort versus tacrolimus and methotrexate in the replication cohort ($P < .001$). The 2 cohorts differed in time: The test cohort was transplanted between 2000 and 2005,

Table 1
Patient Characteristics for Test and Replication Cohorts

	Test Cohort (n = 210)	Replication Cohort (n = 250)	*P*
No. of centers	63	71	
Recipient age at HCT, yr			
Median (range)	45 (6-75)	48 (6-70)	.20
0-19	18 (8)	9 (4)	.43
20-39	61 (29)	73 (29)	
40-59	105 (50)	131 (52)	
≥60	26 (12)	37 (15)	
Recipient sex			.79
Female	99 (47)	121 (48)	
Recipient race			<.001
White	197 (94)	196 (78)	
Nonwhite	10 (5)	9 (4)	
Unknown	3 (1)	45 (18)	
Karnofsky/Lansky score before HCT			<.001
90-100	74 (35)	96 (38)	
<90	112 (53)	150 (60)	
Missing	24 (11)	4 (2)	
Recipient BMI			.43
Underweight	11 (5)	7 (3)	
Normal weight	67 (32)	67 (27)	
Overweight	74 (35)	95 (38)	
Obese	58 (27)	81 (33)	
Disease			<.001
AML	96 (46)	23 (9)	
ALL	37 (18)	181 (72)	
CML	35 (17)	0	
MDS	42 (20)	46 (18)	
Disease status at HCT			.001
Early	82 (39)	141 (56)	
Intermediate	43 (20)	36 (14)	
Advanced	85 (40)	73 (29)	
Median time from diagnosis to HCT, mo (range)	9 (1-194)	6 (<1-177)	<.001
Donor age at HCT, yr			
Median (range)	36 (20-60)	30 (19-58)	<.001
18-29	58 (28)	121 (48)	<.001
30-39	81 (39)	71 (28)	
40-49	57 (27)	43 (17)	
≥50	14 (7)	15 (6)	
Donor race			<.001
White	187 (89)	186 (74)	
Nonwhite	12 (6)	12 (5)	
Unknown	11 (5)	52 (21)	
Donor sex			<.001
Female	99 (47)	75 (30)	
Donor–recipient sex match			<.001
Male–male	101 (48)	89 (36)	
Female–male	61 (29)	80 (32)	
Male–female	10 (5)	37 (15)	
Female–female	38 (18)	38 (15)	
Missing	0	6 (2)	
Donor–recipient CMV status			<.001
+/+	28 (13)	49 (20)	
−/+	18 (9)	24 (10)	
+/−	75 (36)	86 (34)	
−/−	61 (29)	86 (34)	
Missing	28 (13)	5 (2)	
Median $CD34^+$ cell dose, $\times 10^6$/kg (range)	6.6 (2.3-16.8)	n/a	
Conditioning intensity			.001
Myeloablative	148 (70)	197 (79)	
RIC	26 (12)	37 (15)	
NMA	36 (17)	16 (6)	
TBI use in conditioning			<.001
Yes	116 (55)	87 (35)	
Myeloablative (≥5 Gy single dose or ≥8 Gy fractionated)	90 (43)	70 (28)	
GVHD prophylaxis			<.001
Tac+MMF±others	20 (10)	38 (15)	
Tac+MTX±others	57 (27)	155 (62)	
Tac±others	7 (3)	24 (10)	
CSA+MMF±others	34 (16)	14 (6)	
CSA+MTX±others	72 (34)	12 (5)	
CSA±others	10 (4)	2 (<1)	
Others	7 (3)	4 (2)	

(*continued*)

Table ?1 (*Continued*)

	Test Cohort (n = 210)	Replication Cohort (n = 250)	*P*
Missing	3 (1)	1 (<1)	
ATG or alemtuzumab use			<.001
ATG alone	37 (18)	94 (38)	
Alemtuzumab alone	6 (3)	6 (2)	
No ATG or alemtuzumab	164 (78)	150 (60)	
Missing	3 (1)	0	
Year of HCT			<.001
2000-2001	111 (53)	0	
2002-2003	74 (35)	0	
2004-2005	25 (12)	2 (<1)	
2006-2007	0	0	
2008-2009	0	139 (55)	
2010-2011	0	109 (44)	

Values are n (%) unless otherwise defined. BMI indicates body mass index; CML, chronic myeloid leukemia; CMV, cytomegalovirus; CSA, cyclosporine; MMF, mycophenolate mofetil; MTX, methotrexate; NMA, nonmyeloablative; RIC, reduced-intensity conditioning; Tac, tacrolimus; TBI, total body irradiation.

whereas replication cohort patients were transplanted from 2004 to 2011. Cumulative incidence of grades II to IV acute GVHD at day 100 was significantly higher in the test cohort compared with the replication cohort (53%[95% confidence interval {CI},46% to 60%]versus40%[95% CI,34% to 46%], P = .006), but gradesIIIto IV acute GVHD at day 100 was not significantly different (23%[95% CI,18% to 29%] versus16%[95% CI,11% to 21%], P =.06), and nor was chronic GVHD was not significantly different at 1 year (51%[95% CI,44% to 57%]versus43%[95% CI,37% to 50%], P = 0.13). Other donor characteristics, including the distribution of body mass index, were not different between the test and replication cohorts.

Basal Cytokine Levels in Healthy Donors

Serum and plasma circulating cytokine and adipokine levels were measured among healthy donors with available samples. Median levels and ranges for IL-6, IL-1β, TNF-α, ST2, leptin, and adiponectin are presented in Table 2. There was wide variability within each cytokine measurement and significant differences between the test cohort and replication cohort for IL-1β and TNF-α. The median values for IL-1β and TNF-α were significantly different between the test and replication cohorts (P < .001 for both). Circulating cytokine levels for the test and replication cohorts and the quartile cutpoints used for analysis are shown in Table 2.

Test Cohort Outcomes

For grades II to IV acute GVHD, after adjusting for conditioning intensity, year of HCT, GVHD prophylaxis, disease status, ATG/alemtuzumab use, and disease, there were no significant associations with any of the cytokines or adipokines tested. When grades III to IV acute GVHD was considered, adjusting for the same variables as grades II to IV as well as donor sex, no significant association was seen with IL-6 (P = .14), TNF-α (P = .75), ST2 (P = .37), adiponectin (P = .38), or leptin (P = .11). However, there was a borderline overall association with IL-1β (P = .01), and the highest quartile of IL-Iβ was associated with more than a 3-fold increased risk for grades III to IV acute GVHD, nearly reaching statistical significance (P = .01) (Figure 1A, Table 3). A clear dose–response relationship, based on IL-1β quartiles, was not observed.

The models for chronic GVHD were adjusted for conditioning intensity, year of HCT, ATG/alemtuzumab use, disease, donor–recipient sex match, and Karnofsky performance score. Donor TNF-α serum concentration was associated with a decreased risk for chronic GVHD (P = .006) (Figure 2A, Table 4), but none of the other cytokines or adipokines had a significant association with this endpoint. In adjusted models examining OS, DFS, TRM, and relapse, no significant associations were seen with any of the tested cytokines or adipokines.

Table 2
Donor Circulating Cytokine Levels in the Test and Replication Cohorts

Cytokine	Test Cohort	Replication Cohort	*P*
IL-1β, pg/mL			
Mean	529	5.7	<.001
Median (range)	4.8 (0-13,168)	1.4 (0-242.3)	<.001
Quartile 1	1.8	.6	
Quartile 2	4.8	1.4	
Quartile 3	32.9	3.8	
Quartile 4	13,168	242.3	
IL-6, pg/mL			
Mean	519		
Median (range)	1.2 (0-20,454)		
Quartile 1	.5		
Quartile 2	1.2		
Quartile 3	11		
Quartile 4	20,453		
TNF-α, pg/mL			
Mean	104	2.4	<.001
Median (range)	4.6 (0-9028)	1.7 (0-22.8)	<.001
Quartile 1	2.8	1.1	
Quartile 2	4.6	1.7	
Quartile 3	10.6	2.6	
Quartile 4	9028	22.8	
Leptin, pg/mL			
Mean	7051		
Median (range)	3592 (73-75,902)		
Quartile 1	1590		
Quartile 2	3592		
Quartile 3	7144		
Quartile 4	75,902		
ST2, pg/mL			
Mean	14,533		
Median (range)	13,037 (2042-43,620)		
Quartile 1	8443		
Quartile 2	13,037		
Quartile 3	18,813		
Quartile 4	43,620		
Adiponectin, ng/mL			
Mean	13,082		
Median (range)	10,474 (3635-48,575)		
Quartile 1	7199		
Quartile 2	10,474		
Quartile 3	16,646		
Quartile 4	48,575		

Values are mean, median (range), and the upper bounds of each quartile.

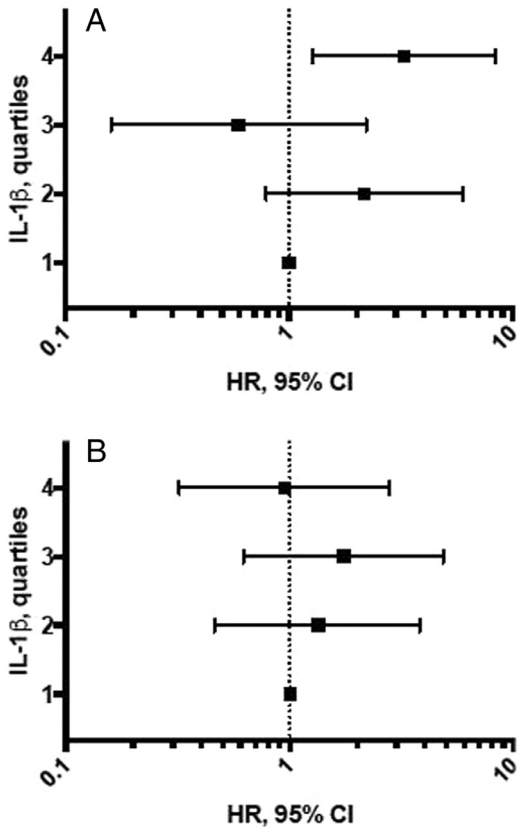

Figure 1. Grades III to IV acute GVHD and IL-1β in the (A) test and (B) replication cohorts. HR indicates hazard ratio.

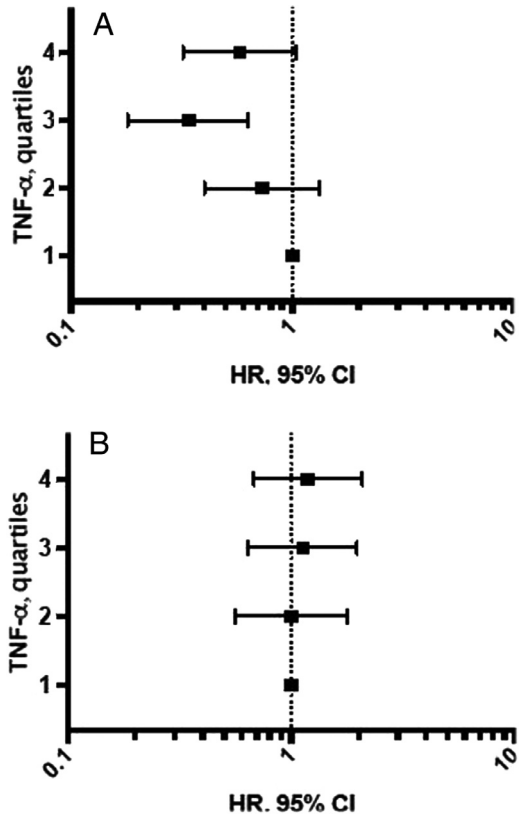

Figure 2. Chronic GVHD and TNF-α in the (A) test and (B) replication cohorts.

Replication Cohort Outcomes

Given the above findings we elected to assess whether these outcomes could be confirmed in a validation cohort. We performed focused analyses to validate the associations between grades III to IV acute GVHD and IL-1β and chronic GVHD and TNF-α. For IL-1β there were no significant associations with grades II to IV or III to IV acute GVHD (P = .49 and P = .56, respectively) (Figure 1B), and there were no significant associations with OS (P = .02), DFS (P = .09), TRM (P = .56), relapse (P = .11), or chronic GVHD (P = .96). Similarly, TNF-α did not show significant associations with chronic GVHD (P = .88) (Figure 2B), grades II to IV GVHD (P = .02), grades III to IV acute GVHD (P = .49), OS (P = .58), DFS (P = .89), TRM (P = .85), or relapse (P = .37).

DISCUSSION

This study was among the first to explore the transferability of donor biologic characteristics into the recipient in a large, heterogeneous, and multicenter cohort. We attempted to identify whether donor proinflammatory cytokines or adipokines were associated with GVHD or other outcomes after HCT. Despite promising findings in the test cohort, with the positive association between IL-1β levels and grades III to IV acute GVHD, we were unable to confirm this in the replication cohort. Similarly, the inverse relationship identified between TNF-α levels and chronic GVHD in the test cohort was not confirmed in the replication cohort.

Despite the inconsistent results between the test and replication cohorts, the initially positive results from the test cohort raise interest in the possibility of transferability of biologic properties from donor to recipient, such as inflammation. In support of this it has clearly been shown that older HCT donor age is associated with inferior outcomes [15] and older age, outside of HCT, is associated with increased levels of inflammation [16] This further raises the question of whether more favorable donor characteristics could be transferred from donor to recipient to induce desired HCT outcomes. Donor statin treatment was protective against acute

Table 3
IL-1β and Grades III to IV Acute GVHD Risk in the Test Cohort

IL-1β quartile	No. of Subjects	Event	Hazard Ratio	95% CI	P
Overall*	175				.01
1	47	8	1.00		Ref
2	45	13	2.17	.79-5.98	.13
3	39	4	.59	.16-2.21	.44
4	44	17	3.26	1.27-8.38	.01

* Adjusted for conditioning intensity, year of HCT, GVHD prophylaxis, disease status, ATG/alemtuzumab use, disease, and donor sex.

Table 4
TNF-α and Chronic GVHD Risk in the Test Cohort

TNF-α quartile	No. of Subjects	Event	Hazard Ratio	95% CI	P
Overall*	187				.006
1	47	28	1.00		Ref
2	48	24	.73	.40-1.33	.30
3	47	25	.34	.18-.63	.0005
4	45	28	.58	.32-1.04	.0685

* Adjusted for conditioning intensity, year of HCT, ATG/alemtuzumab use, disease, sex match, and Karnofsky score.

GVHD after HCT [17]. This suggests that the immune-altering properties of statins are transferred from donor to recipient and persist over time. Although our findings were not consistent across cohorts, we would have expected that large and significant trends among the cytokines investigated in this study would be detectable. Despite these results it remains possible that other donor biomarkers or donor factors, such as immune cell phenotype or function, may influence inflammation and, hence, GVHD risk in the recipient. Donor samples were obtained before G-CSF administration, and it is possible that donor inflammation and circulating cytokine levels would be altered by G-CSF and that this time point may be associated with post-HCT outcomes. Additionally, it is also possible that a single time point does not fully or accurately represent donor biology, and thus our analyses are missing important trends in donor health or inflammation that are not completely captured by a lone blood draw. Further investigation of the augmentation of recipient outcomes through donor characteristics or pharmacologic interventions within donor populations remain important questions for further exploration from the CIBMTR cohort and others.

This study is limited by several factors. There is significant variability between individuals within each cytokine we tested as well as variability between the test and replication cohorts. Of note, the test cohort used serum and the replication cohort was plasma. Although the plasma samples are likely to be slightly more dilute because of the anticoagulant in the collection tubes, it is difficult to understand whether this accounts for the differences we observed between the 2 cohorts. Previous studies have shown the reproducibility of cytokine levels in paired serum and plasma samples [12]. Importantly, the variation in the 2 cohorts does not appear to be due to technical differences, because all samples were run in the same Clinical Laboratory Improvement Amendments–certified laboratory by the same technician, and the Luminex products are calibrated according to the National Institute for Biological Standards and Control. Despite slight differences in the test and validation cohort, we would anticipate that by using the quartile assignments for the analysis, the differences in the magnitude of cytokine levels would not lead to inconsistent findings between the 2 groups.

There were also significant differences in some characteristics of the test and replication cohorts, primarily because of limited availability of donor samples, including disease, year of transplant, GVHD prophylaxis, and use of total body irradiation and ATG, all of which may have influenced the differences in the proportion of recipients experiencing GVHD but are not expected to lead to differences in the distribution of donor cytokine levels.

Other weaknesses include a lack of comprehensive clinical and health data for the donors, which may also explain observed differences. It is unknown what underlying health conditions they had, what medications they were taking, or what their current lifestyle practices were. Cytokines were measured at a single point in time and may not be representative of each individuals' overall inflammatory status over a prolonged time trajectory. More specifically, it is conceivable that the prospect of PBSC donation may differentially affect donors and hence may influence the cytokine levels before collection. Because this study specifically focused on recipients of PBSC allo-HCTs, donors were treated with high doses of G-CSF before cell donation, and although the reported cytokine levels were from before G-CSF exposure, donor systemic inflammation and subsequent recipient outcomes may have been altered by this exposure. It may be more useful to examine measures that would not be affected by acute exposures and may reflect the chronic pro- or anti-inflammatory status of the donor. One such measure includes a recently described gene expression profile, termed the conserved transcriptional response to adversity, characterized by increased expression of multiple proinflammatory genes and decreased expression of genes involved in type I IFN antiviral response and in IgG antibody synthesis [18]. The conserved transcriptional response to adversity has been associated with socioeconomic status, chronic stress, and, when upregulated in the HCT recipient, with inferior HCT outcomes [19-21]. This profile represents an additional pathway for exploring the transferability of donor biology to the recipient.

Overall, this study did not show a consistently significant association between a single time point assessment of donor inflammatory status, represented by a small group of proinflammatory cytokines and adipokines, and GVHD or other post-HCT outcomes. However, there remain potentially novel and important avenues for exploration of the transplantability of donor biologic characteristics that could be more thoroughly investigated prospectively through additional evaluation of donor health and inflammatory status at multiple time points before transplant, including assessment after use of G-CSF, and with more comprehensive data on donor medications and health comorbidities.

ACKNOWLEDGMENTS

The authors thank Michael Ehrhardt of the UMN Cytokine Reference Laboratory for expert technical assistance.

Financial disclosure: Supported in part by the Children's Cancer Research Fund, Minneapolis, Minnesota (to L.M.T.) and the National Marrow Donor Program (to L.M.T.).

Conflict of interest statement: There are no conflicts of interest to report.

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