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Brief Article

Metabolic Complications Precede Alloreactivity and Are Characterized by Changes in Suppression of Tumorigenicity 2 Signaling

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A B S T R A C T

New-onset post-transplantation diabetes mellitus (PTDM) occurs commonly after allogeneic hematopoietic cell transplantation (HCT) and is associated with inferior survival. We hypothesize that PTDM and nonrelapse mortality (NRM) are related to IL-33/suppression of tumorigenicity 2 (ST2) signaling and that soluble ST2 (sST2) levels will predict PTDM diagnosis. sST2 was measured at engraftment and day +30 in 36 euglycemic HCT recipients followed prospectively for PTDM (cohort 1). Results were confirmed in a validation cohort of 26 patients without pre-existing diabetes analyzed retrospectively for PTDM (cohort 2). Twelve patients with established diabetes before HCT were analyzed in cohort 3. When compared with recipients without PTDM, patients developing PTDM (n = 24) from cohort 1 had elevated sST2 levels at engraftment ($P = .02$) and at day +30 ($P < .01$). Cohort 2 confirmed this finding at engraftment ($P = .01$). Cohort 3 patients with pretransplantation diabetes had higher sST2 at engraftment than patients maintaining euglycemia after HCT from cohort 2 ($P = .03$). Multivariate analysis of cohorts 1 and 2 showed high engraftment sST2 predicted increased PTDM and NRM risk, independent of conditioning and grades 3 to 4 acute graft-versus-host-disease. sST2 was elevated in PTDM, indicating a relationship between glucose homeostasis and the IL-33/ST2 axis after transplantation. Correction of metabolic complications may decrease sST2 and improve NRM.

INTRODUCTION

New-onset post-transplantation diabetes mellitus (PTDM) develops in approximately 60% of patients after allogeneic hematopoietic cell transplantation (HCT) [1,2]. Pre-existing diabetes mellitus (DM), new-onset PTDM, and severe acute graft-versus-host disease (aGVHD) are all associated with inferior survival after HCT [1-4]. Glucose homeostasis and aGVHD are affected by IL-33 binding to its receptor, suppression of tumorigenicity 2 (ST2) [5,6]. Soluble ST2 (sST2), a validated predictor of refractory aGVHD and nonrelapse mortality (NRM), acts as a decoy receptor, sequestering excess IL-33 [7,8]. In nontransplantation animal models, IL-33 promotes the development of ST2$^+$ regulatory T cells (Tregs) in visceral adipose tissue, which can be either beneficial or detrimental for obesity- and age-induced insulin resistance, respectively [5,9-11]. We hypothesize that PTDM is related to NRM via IL-33/ST2 dysregulation and that sST2 will predict PTDM diagnosis independently of aGVHD.

METHODS

After providing consent, 74 patients (≥18 years old) with hematologic malignancies undergoing myeloablative or reduced-intensity conditioning followed by related or unrelated donor transplantation were accrued into an institutional review board–approved biomarker study. Graft-versus-host disease (GVHD) prophylaxis consisted of calcineurin inhibitor and either methotrexate or mycophenolate mofetil. Grading of aGVHD followed standard guidelines [12]. Patients were separated into 3 cohorts based on the diabetes diagnosis method. Individuals were monitored for 100 days after day 0.

Cohort 1 (training cohort) consisted of 36 patients without pre-existing diabetes, confirmed by history and a pretransplantation fasting blood sugar (FBS) of <126 mg/dL. Patients had weekly FBS drawn and were followed

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Table 1
Baseline Characteristics of Patients Undergoing HCT (n = 74)

Variable	Cohort 1 (Training) n = 36 (%)	Cohort 2 (Validation) n = 26 (%)	Cohort 3 (DM) n = 12 (%)	*P* Value
Age, median (range), yr	44 (21-65)	49 (31-70)	52 (26-65)	.03
Male	17 (47)	13 (50)	6 (50)	NS
BMI				
18.5-24.9	8 (22)	8 (31)	1 (8)	
25.0-29.9	19 (53)	5 (19)	2 (17)	
> 30.0	9 (25)	13 (50)	9 (75)	
CMV serostatus: donor/recipient				
Negative/negative	11 (30.5)	4 (16)	1 (8)	
Negative/positive	10 (28)	7 (27)	6 (50)	
Positive/negative	4 (11)	3 (11)	3 (25)	
Positive/positive	11 (30.5)	12 (46)	2 (17)	
Standard-risk disease*	18 (50)	12 (46)	6 (50)	NS
Ablative HCT	27 (75)	11 (42)	7 (58)	.03
Related donor	21 (58)	21 (81)	8 (67)	NS
Peripheral blood graft	24 (67)	21 (81)	10 (83)	NS
HLA matched	32 (89)	24 (93)	10 (83)	NS
CSA + methotrexate prophylaxis	24 (67)	11 (42)	6 (50)	NS
PTDM	24 (67)	15 (58)	NA	
Grade 2-4 aGVHD	28 (78)	20 (77)	9 (75)	NS
Grade 3-4 aGVHD	7 (19)	3 (12)	3 (25)	NS

BMI, body mass index; CMV, cytomegalovirus; CSA, cyclosporin A; NS, non-significant, $P > 0.05$.
* Standard risk disease is defined by acute leukemia in first or second complete remission, chronic myeloid leukemia in chronic phase 1, myelodysplastic syndrome without excess blasts. All others were considered high-risk disease.

prospectively for the development of PTDM, defined as the first FBS ≥126 mg/dL or random blood sugar ≥ 200 mg/dL. Cohort 2 (validation cohort) consisted of 26 patients with no history of diabetes who were retrospectively analyzed for PTDM diagnosis, defined as random blood sugar ≥200 mg/dL. Patients with established DM before HCT (n = 12) were analyzed in cohort 3.

Blood specimens were prospectively collected and processed at neutrophil engraftment (absolute neutrophil count ≥ $.5 \times 10^9$/L for 3 days) and day +30. Cryopreserved serum samples were shipped to the Paczesny Laboratory for batch analysis of sST2 using an enzyme-linked immunosorbent assay (quantikine kit, R&D Systems, Minneapolis, MN) [13].

Categorical and continuous variables were compared with the chi-square and Mann-Whitney *U* test, respectively. Mean differences between the 3 groups were examined with a 1-way ANOVA. Univariate (Mann-Whitney *U* test) and multivariate analysis (Cox/competing risks regression) were conducted to test for an association between PTDM and sST2 levels. sST2 was analyzed as both a continuous and nominal variable. To establish a cutoff for a high or low level of sST2, quartiles were examined. After reviewing the distribution of sST2 measurements among patients with or without PTDM, the 75th percentile or higher was chosen as the cutoff for multivariate analysis. Cumulative incidence curves were created to estimate PTDM and NRM, considering death and malignancy relapse as competing risks, respectively. Groups were compared with the Cox/competing risks regression. Covariates for the regression analyses were selected a priori. Myeloablative conditioning and grades 3 or 4 aGVHD were included in the final model as these factors are known to influence both sST2 and survival, respectively [4,7]. Data were analyzed with IBM SPSS Statistics, version 24 (IBM Corp, Armonk, NY) and R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). *P* values were 2-tailed and considered significant at $P \leq .05$.

RESULTS

Table 1 defines characteristics of the 3 groups. In cohort 1, 24 (67%) patients developed new-onset PTDM within 100 days of transplantation. Engraftment, grades 2 to 4 aGVHD, and steroid use were similar between patients developing PTDM and those who maintained euglycemia (Table 2). However, patients with PTDM developed aGVHD and were started on steroids earlier than patients without PTDM. PTDM was diagnosed before grades 2 to 4 aGVHD and steroid initiation in 13 (54%) and 16 (67%) patients, respectively. These data suggest that dysregulation of immune-signaling pathways may promote metabolic complications, which can presage clinical aGVHD. When compared with recipients without PTDM, patients developing PTDM in cohort 1 had elevated sST2 levels at engraftment and on day +30 (Table 2). As expected, higher sST2 levels were associated with aGVHD severity (median, 103 ng/mL versus 24 ng/mL at engraftment; $P < .01$ and median, 175 ng/mL versus 40 ng/mL at day +30; $P = .02$ [grade 3 to 4 versus grade 0 to 2 aGVHD, respectively]). We were concerned that steroid exposure could confound the sST2 and PTDM comparisons. In a stratified analysis performed in patients with aGVHD treated with steroids (n = 26), sST2 remained elevated only in patients developing PTDM (n = 19) at engraftment (median, 50 ng/mL versus 15 ng/mL; $P = .03$) and at day +30 (median, 104 ng/mL versus 17 ng/mL; $P < .01$). The remainder of our sST2 research focused on engraftment samples.

In the validation cohort, 15 (58%) patients developed new-onset PTDM within 100 days of transplantation. Similar to cohort 1, between patients with and without PTDM, there were no differences in engraftment, grades 2 to 4 aGVHD, or

Table 2
Clinical Characteristics of Cohort 1 (n = 36) stratified for PTDM Diagnosis

Variable	No PTDM n = 12 (%)	PTDM n = 24 (%)	*P* Value
Time to neutrophil engraftment, median (range), d	+19 (15-26)	+19 (12-27)	NS
Time to PTDM, median (range), d	NA	+24 (7-100)	
Grade 2-4 aGVHD	8 (67)	20 (83)	NS
Time to aGVHD, median (range), d	+31 (16-47)	+20 (7-93)	.02
Steroid use	8 (67)	18 (75)	NS
Time to steroids, median (range), d	+41 (23-91)	+22 (5-87)	.03
sST2 at engraftment, median (range), ng/mL	20 (8-68)	40 (6-215)	.02
sST2 at d +30, median (range) ng/mL	22 (9-108)	102 (6-272)	<.01