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Association between Thymic Function and Allogeneic Hematopoietic Stem Cell Transplantation Outcome: Results of a Pediatric Study

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Robust T cell function recovery has been shown to be crucial in determining allogeneic hematopoietic stem cell transplantation (HSCT) outcome, and there is growing evidence that the thymus plays a central role in regulating this process. We performed a long-term analysis of the role of thymic activity recovery in a population of pediatric patients undergoing allogeneic HSCT by signal joint T cell receptor excision circle (sjTREC) quantification. In this study, characterized by a long-term follow-up (median, 72 months), we found patients with higher levels of sjTRECs before transplantation had a statistically significant reduced risk of death compared with patients with lower values (relative risk, .31; 95% confidence interval, .30 to .32; $P = .02$), showing this different outcome was mainly related to a reduction of relapse incidence (14% versus 43%, $P = .02$). Unlike previous reports, we observed no correlation between sjTREC levels and lymphocyte recovery. Moreover, we confirmed that only graft-versus-host disease influenced thymic activity after transplantation. In conclusion, our results suggest an association between pretransplantation thymic activity and the long-term outcome of pediatric patients undergoing HSCT, mainly through a reduction of relapse opportunities.

INTRODUCTION

Allogeneic hematopoietic stem cells transplantation (alloHSCT) is 1 of the best therapeutic options available for pediatric patients affected by various malignant diseases and other nonmalignant disorders involving the hematopoietic system [1]. T lymphocyte function recovery is a crucial event in determining the prognosis of patients undergoing alloHSCT because its prolonged impairment may be related to the occurrence of infectious complications and, in the malignant setting, also to the recurrence of primary disease [2,3].

T cell recovery after alloHSCT typically evolves throughout 2 distinct phases, called thymus-independent, or early phase, and thymus-dependent, or late phase. The thymus-independent phase consists of the peripheral expansion of mature T cells transferred to the patient with the graft [4,5]. The thymus-dependent phase consists of the generation of new naive T cells from the donor-derived hematopoietic progenitors occurring in the recipient's thymus. The thymus-dependent phase accounts for the most durable reconstitution of the T cell compartment, generates T cell receptor repertoire diversity [6], and requires a functionally active thymus [7].

Thymic function can be evaluated through the evaluation of the signal joint T cell receptor excision circles (sjTRECs) by quantitative PCR. sjTRECs are episomal DNA fragments resulting from the deletion of the T cell receptor δ region during T cell receptor α locus rearrangement. Because they cannot replicate and are not duplicated, they are diluted out during cell division, allowing a direct evaluation of recent thymic output [8,9].

Previous studies explored the relationship between sjTREC levels and the kinetics of the phenotypic and functional changes in peripheral T cells after alloHSCT, showing a direct correlation between sjTREC levels and the percentage

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of naive T cells resulting from the thymus-dependent recovery pathway in both adult [10,11] and pediatric [10-12] patients. sjTREC levels have also been associated with major parameters affecting the transplantation outcome, such as the incidence of acute and chronic graft-versus-host disease (GVHD) [13,14], opportunistic infections [7,13], and relapse [15,16], but all these studies focused on a single parameter, in a single setting at a single time point [17], and in mixed (pediatric and adult) populations. In this study, we conducted a long-term comprehensive analysis of the impact of sjTRECs on main transplantation outcome variables in a homogenous pediatric population undergoing alloHSCT.

METHODS

Patients

The study population included 57 patients (38 males and 19 females) aged from 0 to 22 years (median age, 9 years) who underwent alloHSCT between April 2006 and October 2008 at our center. To exclude possible bias related to a too-short observation period, analyses were performed when most patients reached a median follow-up of over 5 years. The Institutional Committee on Medical Ethics approved this study, and patients or their legal representatives provided informed consent. Patient characteristics, conditioning regimens, hematopoietic stem cell sources, donor characteristics, and GVHD prophylaxes are summarized in Table 1.

Donor selection and HLA typing were performed according to the Italian Bone Marrow Donor Registry Standard of Practice. In the analyses, total nucleated cell and $CD34^+$ cell values were expressed in percentiles and quartiles according to their non-Gaussian distribution. Pretransplantation comorbidities were scored according to a previously reported classification for pediatric patients [18]. The patients underwent clinical and hematological post-transplantation assessments according to our center's policy. Complete blood counts were performed daily until hematological recovery, twice a week until day +100, and according to patients' clinical conditions thereafter.

Acute and chronic GVHD were diagnosed and classified according to previously reported criteria [19,20]. To monitor patients for viral complications, cytomegalovirus, Epstein-Barr virus, and adenovirus PCR were performed weekly on peripheral blood.

Table 1
Patient and HSCT Characteristics

Characteristic	Subcategory	n	Percent
Sex	Male	38	67
	Female	19	33
Disease	ALL	23	40
	AML	8	14
	Inborn errors	6	10
	Solid tumors	6	10
	Lymphoma	5	9
	MDS and JMML	4	7
	HLH	2	3.5
	SAA	2	3.5
	CML	1	1
Phase*	Early	8	17
	Advanced	39	83
Comorbidity score (18)	0	44	79
	1-2	13	23
	3+	0	
Conditioning regimen	TBI based	31	54
	Bu based	13	23
	Others	13	23
HSC source	BM	46	81
	CB	8	14
	PBSC	3	5
Donor	Sibling	21	37
	MUD	17	30
	MMUD	11	19
	CB	8	14
GVHD prophylaxis	CyA-MTX-ATG	27	48
	CyA	12	21
	CyA-MTX	8	14
	CyA-ATG-MMF	4	7
	CyA-ATG-PDN	3	5
	Others	3	5

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; MDS, myelodysplasia; JMML, juvenile myelomonocytic leukemia; HLH, hemophagocytic lymphohistiocytosis; SAA, severe aplastic anemia; CML, chronic myelogenous leukemia; TBI, total body irradiation; Bu, busulfan; BM, bone marrow; CB, cord blood; PBSC, peripheral blood stem cell; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; CyA, cyclosporine; MTX, methotrexate; ATG, antithymocyte globulin; MMF, mycophenolate mofetil; PDN, prednisone.

* For malignant diseases only.

sjTREC Frequency Evaluation

The day before starting the conditioning regimen, on days 90 ± 7, 180 ± 7, and 365 ± 7, patients were evaluated for sjTREC frequency according to previously reported methods [21,22] on peripheral blood mononuclear cells (PBMC) by real-time quantitative PCR (TaqMan Technology, Applied Biosystem, Foster, CA). The primer TREC sequences and probes used were as follows: forward, 5′-TGGTTTTTGTGCCCAC-3′; reverse, 5′-GTGCCAGCTGCAGGGTTT-3′; probe, 5′(FAM) CATAGGCACCTGCACCCCGTGC (TAMRA) P-3′. PCR conditions were as follows: 2 minutes at 50°C, 10 minutes at 95°C followed by 45 cycles of amplification (95°C for 15 seconds, 60°C for 1 minute). To obtain absolute sjTREC quantification, we prepared a standard curve by using 5 different concentrations of a PCR2-1TA plasmid encoding the sjTREC sequence. PCR was performed using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), and data obtained were analyzed using SDS.2 software (Applied Biosystems). sjTREC values are expressed as copy number/100 ng DNA from PBMCs. Because the non-Gaussian distribution of sjTREC values and almost all patients enrolled in this study had median sjTREC values under the median value of age-matched control subjects at all time points, all analyses were performed considering sjTREC percentiles and quartiles of the study population.

Definitions and Outcome Endpoints

The primary endpoint of this study was the assessment of the impact of sjTREC levels on the overall survival (OS) rates in a population of pediatric patients undergoing HSCT. Secondary endpoints were the assessment of sjTREC levels on both transplant-related mortality (TRM) and relapse incidence (RI) and the identification of transplant-related factors able to influence sjTREC levels. OS was defined as the probability of survival irrespective of the disease state at any point in time. If, at the end of the study time, the patient was still alive, data were censored at the last follow-up date.

TRM was defined as the probability of dying without a previous relapse occurrence. If the patient either experienced relapse or was still alive at the end of the study time, data were censored at the relapse date or at last follow-up date, respectively. For malignant diseases, RI was defined as the probability of having had a relapse. If the patient either died without experiencing relapse or was still alive at the end of the study time, data were censored at the date of death or at the last follow-up date, respectively. For malignancies, patients not in a first complete remission at the time of transplant and patients who had previously failed at least 1 first-line treatment were considered to be in an advanced disease phase, whereas all other patients were considered to be in an early disease phase.

Chimerism and Immune Recovery Evaluation

Donor chimerism was determined at +30 ± 7 and +60 ± 7 days after alloHSCT on whole bone marrow mononuclear cells and at +180 ± 7 and +365 ± 7 days on PBMCs by quantitative PCR of informative short tandem repeats in the recipient and donor, according to a previously described method [23]. Absolute lymphocyte numbers were obtained from complete blood count analyses and compared with normal values according to patient age [24]. Lymphocyte recovery was defined as the first of 3 consecutive days with an absolute lymphocyte count over the fifth percentile of normal values for the patient's age. In a subset of patients, we also investigated specific lymphocyte subpopulation recovery at +180 days and +365 days by flow cytometry. Helper T cell ($CD3^+CD4^+$), cytotoxic T cell ($CD3^+CD8^+$), natural killer cell ($CD16^+CD56^+$), and B cell ($CD19^+CD20^+$) recovery was defined as the presence of an absolute number of cells over the fifth percentile of normal values according to patient age [24].

Statistical Analysis

OS was calculated according to the Kaplan-Meier method, and the significance between the observed differences was established by the log-rank test [25]. The multivariate analysis on OS was performed using Cox's method.

TRM and relapse rate were calculated as a cumulative incidence to adjust the analysis for competing risks: relapse and transplant-related death were considered competing risks, respectively. The differences in terms of cumulative incidence were compared using Gray's test. To assess the influence of different transplant-related variables on sjTREC levels, a 2-tailed