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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

Fisher Test was performed. $P < .05$ was considered statically significant. To perform multivariate analyses we selected variables reaching $P < .1$ in the univariate analyses. All statistical analyses were performed using SPSS (IBM Corp., Armonk, NY), NCSS (NCSS PASS, Number Crunched Statistical System, Kaysville, UT), and R 2.5.0 (www.r-project.org) software packages.

RESULTS

sjTREC Frequency

Median sjTREC values were 16 (range, 0 to 1684), 1 (range, 0 to 160), 14 (range, 0 to 553), and 201 (range, 0 to 1006) sjTREC copies/100 ng DNA before HSCT, at day +90, at day +180, and at day +365, respectively. To identify transplant-related factors associated with the frequency of sjTRECs, we evaluated the impact of different variables on median sjTREC values, before HSCT and then at different time points (Table 2).

Table 2
sjTREC Frequency

Characteristic	No. of Patients with sjTREC Level < Median Value of the Study Population	No. of Patients with sjTREC Level > Median Value of the Study Population	*P*
Pre-HSCT (n = 57)			
Age			.04
0-5 yr (n = 15)	6 (40%)	9 (60%)	
6-8 yr (n = 11)	3 (27%)	8 (73%)	
9-14 yr (n = 16)	8 (50%)	8 (50%)	
>14 yr (n = 15)	8 (53%)	7 (47%)	
Disease			.03
Malignant (n = 47)	28 (60%)	19 (40%)	
Nonmalignant (n = 10)	2 (20%)	8 (80%)	
Comorbidities (18)			.21
0 (n = 44)	21 (48%)	23 (52%)	
1-2 (n = 13)	9 (70%)	4 (30%)	
Disease phase*			.005
Early (n = 8)	1 (12%)	7 (88%)	
Advanced (n = 39)	27 (69%)	12 (31%)	
Time from diagnosis to HSCT			.57
<6 mo (n = 25)	16 (64%)	9 (36%)	
>6 mo (n = 22)	12 (54%)	10 (46%)	
Day +90 (n = 57)			
ATG			.02
Yes (n = 37)	25 (68%)	12 (32%)	
No (n = 20)	5 (25%)	15 (75%)	
Viral infection			.01
Yes (n = 30)	21 (70%)	9 (30%)	
No (n = 27)	9 (33%)	18 (67%)	
Day + 180 (n = 57)			
Grades II-IV acute GVHD			.03
Yes (n = 21)	15 (71%)	6 (29%)	
No (n = 36)	16 (44%)	20 (56%)	
Day + 365 (n = 43)			
Age			.03
0-5 yr (n = 9)	3 (33%)	6 (64%)	
6-8 yr (n = 10)	3 (30%)	7 (70%)	
9-14 yr (n = 14)	7 (50%)	7 (50%)	
>14 yr (n = 10)	9 (90%)	1 (10%)	
Chronic GVHD			.02
Yes (n = 6)	6 (100%)	0	
No (n = 37)	16 (43%)	21 (57%)	
Viral infection			.03
Yes (n = 24)	16 (67%)	8 (33%)	
No (n = 19)	6 (32%)	13 (68%)	

* For malignant diseases only.

Overall Survival

As of March 2014, the median follow-up time of patients who were still alive was 72 months (range, 42 to 90). The OS rate at 7 years of the entire study population was 70% (95% confidence interval [CI], 58% to 82%). We found a statistically significant relationship between sjTREC frequency before transplantation and 7-year OS. Patients with sjTREC values below the 50th percentile of the study population before HSCT had an OS rate of 56% (95% CI, 38% to 73%), whereas patients with sjTRECs above the 50th percentile had an OS rate of 85% (95% CI, 71% to 98%) ($P = .02$) (Table 3 and Figure 1). Moreover, before transplantation, it was possible to perform a more extended analysis considering the sjTREC frequency subgrouped into quartiles: patients with sjTREC values in the first, second, third, and fourth quartiles had OS rates of 40%

Table 3
Overall Survival

Variable	n	Events	7-yr OS	95% CI	Log Rank Test, *P*
sjTRECs pre-HSCT					
<50th percentile	30	13	56%	(38-73)	.02
>50th percentile	27	4	85%	(71-98)	
sjTRECs +90 days					.97
<50th percentile	30	9	70%	(54-86)	
>50th percentile	27	8	70%	(52-88)	
sjTRECs +180 days					.1
<50th percentile	29	7	60%	(42-78)	
>50th percentile	25	8	80%	(66-94)	
sjTRECs +365 days					.6
<50th percentile	20	4	77%	(59-95)	
>50th percentile	18	6	83%	(65-100)	
Sex					.035
Male	38	15	60%	(44-76)	
Female	19	2	89%	(75-100)	
Age					.28
0-5 yr	15	5	63%	(36-90)	
6-8 yr	11	4	64%	(36-91)	
9-14 yr	16	2	87%	(71-100)	
>15 yr	15	6	60%	(34-85)	
Disease					.14
Malignant	47	16	65%	(53-81)	
Nonmalignant	10	1	90%	(63-100)	
Comorbidity score					<.0001
Low-risk group	44	7	84%	(83-84)	
Intermediate-risk group	13	10	23%	(0-46)	
Disease phase*					.04
Early	8	0	100%		
Advanced	39	16	60%	(44-76)	
Time between diagnosis and HSCT*					.55
<6 mo	25	7	73%	(56-90)	
>6 mo	22	9	60%	(40-80)	
TBI					.51
Yes	31	8	73%	(57-89)	
No	26	9	65%	(47-83)	
HSC source					.27
BM	46	16	65%	(51-79)	
PBSC	3	1	87%	(63-100)	
CB	8	0	100%		
TNCs					.65
<50th percentile	29	9	69%	(51-87)	
>50th percentile	28	8	71%	(53-89)	
$CD34^+$ cells					.78
<50th percentile	30	9	70%	(54-86)	
>50th percentile	27	8	69%	(51-87)	

TNCs indicates total nucleated cells.
All variables potentially able to influence OS were evaluated: sjTREC levels before alloHSCT, patient's sex, comorbidities, and disease phase showed a statistically significant ($P < .05$) correlation with OS.
* For malignant diseases only.

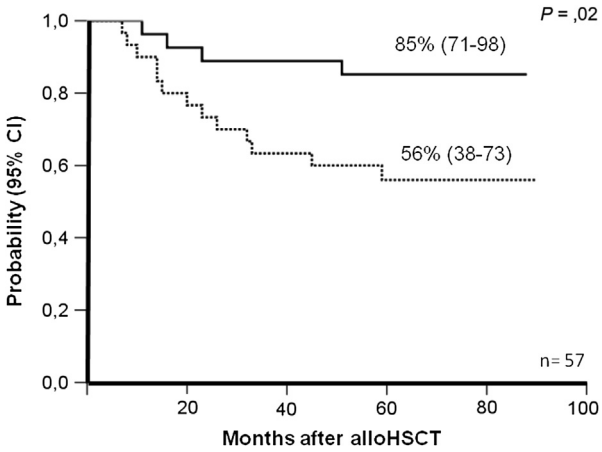

Figure 1. OS according to sjTREC levels. Patients with sjTRECs over the 50th percentile before HSCT (continuous line) showed a statistically significant increased survival rate compared with patients with sjTRECs under the 50th percentile (dotted line) at same time point.

(95% CI, 14% to 65%), 71% (95% CI, 47% to 94%), 87% (95% CI, 69% to 100%), and 83% (95% CI, 63% to 100%) respectively; these differences were statistically significant ($P = .009$).

Considering OS at 2 years, we found a difference according to pre-HSCT sjTREC levels: Patients with sjTREC levels under the median value of the study population had an OS rate of 73% (95% CI, 57% to 89%), whereas patients with sjTREC levels over the median value of the study population had an OS rate of 89% (95% CI, 57% to 89%), although this difference was not statistically significant ($P = .13$). Restricting 2-year OS analysis according to pre-HSCT sjTREC levels only to the cohort of patients affected by malignant diseases, we also highlighted a difference, but it was not statistically significant ($P = .14$).

Female patients showed better OS compared with male patients (89% [95% CI, 75% to 100%] versus 60% [95% CI, 44% to 76%], $P = .035$), and patients in an early disease phase had better OS compared with patients in advanced disease phases (100% versus 60% [95% CI, 44% to 76%], $P = .04$). All other variables investigated in the univariate analysis (Table 3) showed no correlation with OS. In particular, we did not observe a correlation between OS or sjTREC levels at +90, +180, and +365 days after HSCT (Table 3).

To perform multivariate analysis, we selected from among variables listed in Table 3, those reaching $P < .1$ in the univariate analysis (sex, comorbidities, disease phase at HSCT, and pre-HSCT sjTREC levels). In the multivariate analysis, sjTREC levels before transplantation and pre-HSCT comorbidities were the only variables we found to be associated with OS: Patients with higher sjTRECs values showed a statistically significant reduced risk of death compared with patients with lower sjTRECs values (relative risk, .49; 95% CI, .48 to .5; $P = .03$), and patients in the intermediate-risk group (score 1 to 2) according to Smith et al. [18] showed a statistically significant increased risk of death compared with patients in the low-risk group (score 0) (relative risk, 2.5; 95% CI, 2.49 to 2.5; $P = .03$). In multivariate analysis sex and disease phase showed no statistically significant relationship with OS.

Transplant-Related Mortality

The overall TRM was 5% (95% CI, 2% to 16%). In the univariate analysis sjTREC levels before transplantation and sjTREC levels at +90 days did not show any correlation with the TRM. At +180 days from transplant, patients with sjTRECs values under the 50th percentile had a TRM rate of 11% (95% CI, 4% to 32%) versus a TRM rate of 0 of patients with sjTREC values over the 50th percentile. Likewise, at +365 days patients with sjTREC values under the 50th percentile had a TRM rate of 10% (95% CI, 1% to 37%) versus a TRM of 0 in patients with sjTREC values over the 50th percentile. These differences in terms of TRM were not statistically significant ($P = .1$ and $P = .17$, respectively) (Table 4).

Relapse Incidence

For malignant disease, the overall RI was 30% (95% CI, 20% to 46%). sjTREC levels before transplantation were related to relapse. Patients with sjTREC levels below the 50th percentile of the study population relapsed in 43% of cases (95% CI, 28% to 66%), whereas 14% of patients with sjTREC levels above the 50th percentile experienced a relapse (95% CI, 5% to 41%); this difference was statistically significant ($P = .02$) (Table 4 and Figure 2).

Considering sjTREC levels before the transplant subgrouped in quartiles, patients with sjTREC levels in the first, second, third, and fourth quartiles had a relapse in 64% (95% CI, 43% to 95%), 21% (95% CI, 8% to 58%), 14% (95% CI, 4% to 51%), and 14% (95% CI, 2% to 88%) of cases, respectively, and this difference was statistically significant ($P = .01$). sjTREC levels at +90, +180, and +365 days were not related to the recurrence.

Among other variables investigated by univariate analysis, patient gender showed a relationship with RI: Male patients relapsed in 40% (95% CI, 27% to 60%), whereas female patients relapsed in 7% (95% CI, 1% to 47%; $P = .03$) (Table 5). To perform multivariate analysis, we selected, from among the variables listed in Table 5, those reaching $P < .1$ in the univariate analysis (sex and pre-HSCT sjTREC levels) and disease phase at HSCT. In the multivariate analysis sjTREC levels before transplantation were the only variables we found to be statistically associated (relative risk, 0; $P < .0001$) with RI.

Chimerism and Immune Recovery

All patients showed sustained engraftment, and we did not observe any cases of either early or late graft loss. Patients enrolled in the study reached the fifth percentile of normal lymphocyte values for patient age in a median of 70 days (range, 21 to 420), with no differences related to pre-HSCT sjTREC levels: 73 days (range, 25 to 420) for patients with sjTRECs over the 50th percentile before HSCT versus 65 days (range, 21 to 385) for patients with sjTRECs under the

Table 4
TRM Univariate Analysis

Variable	TRM	95% CI	Gray Test, *P*
sjTRECs pre-HSCT			.46
<50th percentile	3%	(0-23)	
>50th percentile	7%	(2-28)	
sjTRECs +90 days			.60
<50th percentile	7%	(2-25)	
>50th percentile	4%	(0-26)	
sjTRECs +180 days			.10
<50th percentile	11%	(4-32)	
>50th percentile	0		
sjTRECs +365 days			.17
<50th percentile	10%	(1-37)	
>50th percentile	0		

sjTREC level showed no statistically significant correlation ($P < .05$) with TRM at any time point considered.

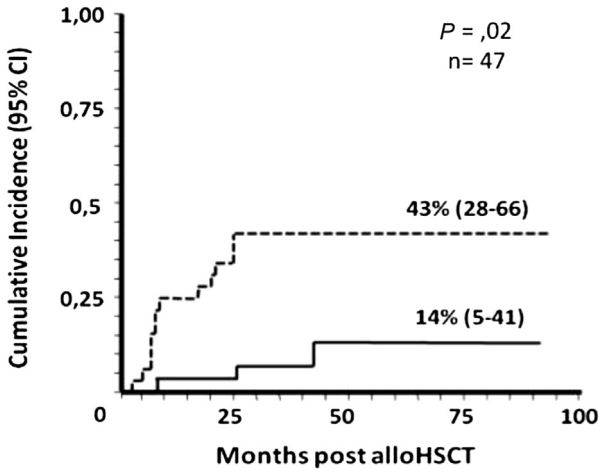

Figure 2. Relapse rate according to sjTREC levels. Patients with sjTRECs over the 50th percentile before HSCT (continuous line) showed a statistically significant reduced relapse rate compared with patients with sjTRECs under the 50th percentile (dotted line) at same time point.

50th percentile before HSCT. Considering lymphocyte subpopulations, the proportion of patients who reached the fifth percentile of normal values for their ages of $CD3^+CD4^+$, $CD3^+CD8^+$, $CD16^+CD56^+$, and $CD19^+CD20^+$ cells was 17%, 65%, 82%, and 60% at day +180 and 70%, 85%, 88%, and 77% at day +365, respectively, with no differences related to pre-HSCT sjTREC levels.

DISCUSSION

T cell function recovery has been shown to be 1 of the most important factors in determining the prognosis of patients undergoing alloHSCT, and the role of the thymus in this process is well established. Previous studies focused on severe combined immunodeficiency disease screening programs in newborns [26] and on the management of patients affected by HIV and undergoing highly active antiretroviral therapy [27] indicated that sjTREC quantification is an easy, sensible, and reliable technique to evaluate immunologic function and also to drive therapeutic interventions in these settings. Although the experience of alloHSCT is more limited, there is growing evidence that sjTREC quantification by PCR is 1 of the easiest and most reliable methods to evaluate thymic activity after alloHSCT as well. This is because, compared with other techniques (ie, flow cytometry), this method offers the advantage of not being influenced by any phenomena that typically occur after transplantation, such as the opportunity of T memory cells to revert into a T naive phenotype, as in case of recurrent herpes virus infection [28]; the possibility of T naive cells to maintain their phenotype while acquiring T memory cells' function [29]; and the maintenance of CD31 expression during $CD4^+$ cell cytokine-driven proliferation [30].

Even though other studies have already shown a correlation between sjTREC levels and the various phases of immune recovery after alloHSCT [10-12] and that patients with a more efficient thymic function show a better prognosis compared with others [13,31], there are still very few studies specifically concerning pediatric patients. Considering aging is a major parameter impacting thymic function [9,32], childhood may be considered an ideal setting to further consolidate these data. In the present study we analyzed in a population of pediatric patients undergoing alloHSCT the role of sjTREC levels on OS and found that patients with more efficient thymic function before transplantation had better long-term OS compared with others. However, sjTREC levels after transplantation, according to our data, did not have any influence on OS at any of the time points considered.

To our knowledge only 2 previous studies specifically investigated the impact of sjTRECs on OS. Clave et al. [17] demonstrated a correlation between pretransplantation sjTREC levels and OS, but even though a high number of cases were reported (n = 102), only sibling recipients were included, the patients' median age was higher, and only pretransplantation sjTREC levels were considered. In 66 pediatric patients who underwent alloHSCT, Olkinuora et al. [33] reported a shorter median survival time for patients with low sjTREC levels at different time points (both before and after alloHSCT) compared with patients with high sjTREC levels, but their study lacked a real survival analysis performed with the Kaplan-Meier method and the follow-up was shorter. We basically confirmed a correlation between sjTREC levels and OS in a more homogeneous and younger population that also included unrelated transplant recipients, and by extending the follow-up to a median time of 72 months, we highlighted that among pediatric patients

Table 5
RI for Malignant Diseases: Univariate Analysis

Variable	n	Events	RI	95% CI	Gray Test, *P*
sjTRECs pre-HSCT					.02
<50th percentile	28	12	43%	(28-66)	
>50th percentile	19	3	14%	(5-41)	
sjTRECs +90 days					.60
<50th percentile	26	7	26%	(14-49)	
>50th percentile	21	8	33%	(18-61)	
sjTRECs +180 days					
<50th percentile	27	10	37%	(15-52)	.34
>50th percentile	20	5	25%	(5-46)	
sjTRECs +365 days					.36
<50th percentile			11%	(3-41)	
>50th percentile			23%	(8-62)	
Sex					.03
Male	34	14	41%	(27-61)	
Female	13	1	8%	(1-50)	
Age					.58
0-5 yr	12	4	33%	(14-69)	
6-8 yr	8	4	50%	(21-92)	
9-14 yr	15	3	20%	(7-55)	
>15 yr	12	4	33%	(15-74)	
Disease phase					.20
Early	8	1	12%	(2-78)	
Advanced	39	14	35%	(22-52)	
Time between diagnosis and HSCT					.67
<6 mo	25	7	28%	(15-52)	
>6 mo	22	8	36%	(21-63)	
HSC source					.29
BM	39	14	36%	(24-55)	
CB	7	1	14%	(2-87)	
PBSC	1	0	0		
Donor					.24
Related	17	7	41%	(23-73)	
Unrelated	30	8	27%	(15-48)	
Acute GVHD					.41
Yes	20	5	25%	(12-53)	
No	27	15	37%	(23-60)	
Chronic GVHD					
Yes	8	2	25%	(7-83)	.57
No	39	13	32%	(20-50)	

Univariate analysis of variables potentially able to influence RI: sjTRECs before the transplantation and patient's sex were statistically related to RI incidence ($P < .05$).

long-term survival is closely related to pre-HSCT sjTREC levels. However, a limitation of our study is that a large proportion of the patients enrolled in our study had acute lymphoblastic leukemia, which tends to undergo relapse in the first months after transplantation; therefore, the correlation between sjTREC levels and OS seems to be less strong in the short term. Moreover, the small number of patients affected by nonmalignant disorders included in the study population might be also responsible for the absence of a statistically significant association between 2-year OS and pre-HSCT sjTREC levels.

To understand whether the mortality reduction we observed was attributable to a reduction of either TRM or RI, we analyzed in the same population the impact of sjTREC levels on these 2 outcome parameters. In line with other authors' findings, we observed a strong correlation between pretransplantation thymic functions and RI [15,16]. However, unlike these authors, who investigated the role of sjTRECs in only 1 specific setting, surprisingly, we did not observe a correlation between post-transplantation thymic activity and RI. This difference might be related to the heterogeneity of our study population that included bone marrow, peripheral blood stem cells, and cord blood recipients. One possible objection to our observations could be that reduced sjTREC frequency before alloHSCT might be related to more intense treatments administered because of a more aggressive disease and that OS and RI differences might only be related to a more advanced disease phase. However, via multivariate analysis, we were able to show how sjTREC levels before transplantation are statistically associated with OS and RI independently from other variables, including the presence of an advanced disease phase. By correlation analysis, we excluded a link between the disease phase at transplantation and the time between diagnosis and HSCT and sjTREC frequency before alloHSCT. In multivariate analysis we found the only other variable associated with OS was the presence of comorbidities as scored by Smith et al. [18]. Unlike previous observations [7,13,14,33,34], we did not observe a relationship between sjTRECs and TRM, probably because of the very low incidence of these complications in our study population related to the lower frequency of comorbidities in young individuals.

Finally, to clarify whether increased OS and reduced RI of patients with higher pre-HSCT sjTREC levels were related to an improved immune recovery, we evaluated the absolute lymphocyte count recovery. Surprisingly, we did not observe any differences between the patients with values over the 50th percentile or patients with values under the 50th percentile. Our data contrast with previous reports [12], but this might be because of our smaller sample size and might be related to the differences in the recovery of different lymphocyte subsets (T, B, natural killer) after HSCT. By analyzing transplant-related factors that influence sjTREC frequency, we confirmed previously reported observations on adults [35] confirming GVHD as 1 of the most limiting factors in determining sjTREC levels after transplantation. However, according to our analysis, sjTREC reductions after HSCT were not statistically correlated with any worsening in terms of OS, TRM, or relapse.

The main weakness of our study is that we analyzed sjTREC frequency on whole PBMCs, whereas other authors performed the same analysis more precisely on selected lymphocyte populations (ie, $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$). Another limitation of our study is that in the series of patients we described, most were affected by acute lymphoblastic leukemia, but no cases of T cell leukemia were included; this may have some consequences in terms of both RI and OS.

In conclusion, our results confirm that thymic function does play an important role in determining the prognosis of pediatric patients undergoing alloHSCT, suggesting an efficient thymic function before transplantation is related to improved long-term OS, mainly through a reduction of relapse opportunities. Obviously, larger and more accurate studies are needed both to confirm these observations and to identify the mechanism driving them to find solutions aimed at improving T cell recovery after alloHSCT.

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