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ORIGINAL ARTICLE

Immune reconstitution after HSCT in SCID—a cohort of conditioned and unconditioned patients

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Abstract

Allogeneic hematopoietic stem cell transplantation (HSCT) is the effective mean of immune restoration in severe combined immunodefiency (SCID). Usually, HSCT without cytoreductive conditioning is attempted. Nevertheless, conditioning procedures are still preferred in a subset of patients. Herein, we describe the immunological outcome in a cohort of conditioned and unconditioned patients, from diagnosis, through transplantation, to follow-up. This retrospective study was conducted on 17 patients with SCID (10 conditioned, 7 unconditioned) who later underwent HSCT. Immune reconstitution was assessed in the post-transplant year by quantification of T cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs), among additional laboratory and clinical evaluations. Unconditioned patients were diagnosed and transplanted earlier. TREC and KREC quantification showed a gradual increase in both groups, with higher levels in the conditioned group. Engraftment percentages differed drastically between groups, favoring the conditioned group. Unconditioned patients were significantly more dependent on intravenous immunoglobulins (IVIGs). One patient from each group succumbed to disease complications. Conditioning demonstrated superior laboratorial outcomes. Patients with unique characteristics (i.e., consanguinity, *Bacillus Calmette–Guérin* vaccination, impaired access to IVIG) may require personalized considerations. The effort to implement secondary prevention of SCID with newborn screening should continue.

Keywords Severe combined immunodeficiency (SCID) · Hematopoietic stem cell transplantation (HSCT) · T cell receptor excision circles (TRECs) · Kappa-deleting recombination excision circles (KRECs) · Conditioning · Primary immunodeficiency (PID)

Introduction

Severe combined immunodeficiency (SCID) is a rare immune disorder of infancy, characterized by a lack of antigen-specific T cell response and concurrent variable B cell function and lymphopenia. SCID syndromes are usually classified and diagnosed by either their genotype or cellular immunophenotype. Genotypes confer disease pathophysiology [cytokine signaling defects (i.e., *JAK3*, *IL2R*), dysfunctional rearrangements (i.e., *RAG1*, *RAG2*), metabolic defects (i.e., *ADA*), and so forth], yet require considerable genetic testing. Immunophenotype (T−, B+/−, NK+/−) enables rapid assumptive bottom-up diagnosis [1]. The presence of dysfunctional T cells can be found in some "leaky" forms of SCID, such as Omenn syndrome or due to maternally engrafted T cells [2]. Early hematopoietic stem cell transplantation (HSCT) has been the mainstay of treatment for SCID, notwithstanding its caveats: rejection, graft-versus-host disease (GVHD), poor B cell reconstitution with subsequent dependence on intravenous immunoglobulin (IVIG) infusions, morbidity, and mortality associated with pre-transplantation chemoablation. The need for conditioning in profound immunodeficiencies is debated—conditioning appeared necessary to achieve superior engraftment, yet it entails substantial adverse effects. Moreover, SCID is a unique HSCT indication in the sense that patients completely lacking T cell immunity may not demand myeloablation before the procedure, especially when human leukocyte antigen (HLA)–matched donors are available. However, a considerable number of patients receive HSCT from donors who are mismatched at some degree or have

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residual T cell immunity, thus warranting chemotherapeutic pre-transplantation conditioning and/or post-procedural GVHD prophylaxis [3]. The donor's compatibility to the recipient is the most important factor dictating the HSCT method and has a significant effect on overall survival. The multitude of variants affecting the transplantation procedure has produced a lack of consensus regarding optimal practice. Centers differ in many aspects of treatment: from their algorithm for donor choice, through their choice to condition (and if so—which protocol to use), to follow-up methods and duration [4].

Immune reconstitution may be evaluated in several ways. Successful engraftment is assessed by lymphocyte chimerism and/or fluorescence in situ hybridization (FISH) in gender-mismatched transplants. It should be noted that full donor chimerism is not expected and probably not necessary [5]. Quantification of immunoglobulins and characterization of lymphocyte subpopulations can further demonstrate immune recovery. In recent years, quantification of T cell receptor excision circles (TRECs) and kappa-deleting excision circles (KRECs) and analysis of the T cell receptor (TCR) and B cell receptor (BCR) repertoires have proven to be the most advanced assays for this purpose [5–7]. TRECs and KRECs are circular episomal excision products of the T and B cell maturation in the thymus and bone marrow, respectively. A highly heterogeneous repertoire of lymphocyte receptors is created in these sites through the variable (V), diversity (D), and joining (J) gene rearrangement processes. In T cell maturation, V, D, and J segments are flanked by recombination signal sequences (RSSs), where recombination activating genes (RAGs) cleave the DNA. V(D)J exons with coding joints (CJs) join and continue transcription and translation into T cell receptors. The "leftover" DNA, fenced by two RSS, fuses to create a signal joint (SJ), thus producing an extrachromosomal circular excision product, a.k.a. SJ-TREC. In B cell maturation, a similar process occurs during the rearrangement of the kappa light chain. Both elements (hereinafter TRECs, KRECs) are stable, do not replicate, and therefore are renowned as markers for new lymphocyte output. Soon after identification, their quantification by means of real-time quantitative polymerase chain reaction (RQ-PCR) has been a part of SCID diagnosis, characterization, and treatment monitoring [8–10].

In this study, we described and characterized the clinical and laboratorial immune reconstitution of our cohort, comprised of different genotypes and donor sources. We examined our data through the prism conditioning—a subgroup of seven unconditioned patients vs. ten conditioned ones.

Methods

Patients

Seventeen consecutive patients with SCID transplanted at the Sheba Medical Center (Tel HaShomer, Israel) between 2008 and 2015 were recruited to this study, after approval of their parents and the institutional review board. Diagnosis was based on clinical findings implying immunodeficiency, family history, immunological evaluation, and genetic testing. HLAs A, B, C, and DR were typed using serological or DNA hybridization methods in order to define donor–recipient compatibility. Patients with matched related donors (MRDs), no evidence of Omenn's syndrome nor substantial maternal engraftment, were assigned for a procedure without conditioning. Patients who failed to fulfil the above underwent cytoreductive protocols. SCID genotype did not affect assignation.

Transplantation

Patients received the transplantation inhouse, at our hemato-oncology unit. Standard prophylactic medications consisted of trimethoprim/sulfametaxozole for *Pneumocystis jiroveci* (PCP), IVIG to maintain IgG level above 6 g/L, acyclovir in the occasion of donor–recipient serologic *cytomegalovirus* (CMV) disparity, and cyclosporine A (CSA) or mycophenolate mofetil (MMF) for GVHD in conditioned patients. GVHD was graded between I and IV according to convention, and the acuteness chronicity limit defined at 100 days. Granulocyte colony-stimulating factor (G-CSF) was given from the day of transplant until neutrophil counts were above 1.0×10^9/L for three consecutive days. The HSCT infusion itself originated from either bone marrow, peripheral blood, or cord blood. Conditioning regimens were based on the European group for blood and marrow transplantation (EBMT) and European Society for Immunodeficiencies (ESID) guidelines for HSCT in PID [11].

Engraftment and immunologic assessment

Neutrophil engraftment was defined after three consecutive days with an absolute count of over 0.5×10^9/L. Lymphocyte engraftment and chimerism were evaluated mainly by analysis of microsatellite variable numbers of tandem repeats, or FISH studies using Y-specific probes in gender-mismatched couples.

Absolute numbers and percentages of lymphocytes were quantified by assessment of cell surface markers using immunofluorescent staining and flow cytometry (Epics V; Beckman Coulter, Hialeah, FL, USA) with antibodies purchased from Beckman Coulter. Serum immunoglobulin concentrations (IgG, IgM, IgA) were measured using standard nephelometry.

TREC and KREC quantifications were performed serially on genomic DNA extracted from patients' peripheral blood mononuclear cells (PBMCs) and determined by RQ-PCR. RQ-PCR reactions were carried out in an Applied Biosystems™ StepOnePlus™, using TaqMan universal PCR master mix (Applied Biosystems™), specific primers, and FAM-TAMRA probes for TRECs and KRECs. Absolute levels in each sample were automatically calculated by comparing the cycle threshold

(Ct) value to a validated standard curve. Age-matched healthy individuals were used as controls, amplification of RNAseP served as a quality control, and Ct thresholds were positioned at similar levels.

Statistical analysis

Statistical analysis was performed using SPSS software for windows, version 22.0 by IBM. p values less than 0.05 on a two-sided test were considered statistically significant. Clinical parameter distributions were tested for normality by the Shapiro–Wilk test. The Mann–Whitney U test and the Wilcoxon signed-rank test were used for continuous variables with a non-normal distribution, and the relationship between categorical variables was evaluated using Fisher's exact test, which was more appropriate than the χ^2 test due to the small sample size. Unless otherwise specified, values are given as mean ± standard deviation (SD).

Results

Patients

From a total of 17 patients, 16 manifested with classical SCID-associated symptoms, such as failure to thrive (FTT), recurrent fevers, and infections. Five patients displayed clinical manifestations of Omenn syndrome. Patient #3, benefitting from having an afflicted elder sibling who underwent genetic workup and HSCT, was diagnosed prenatally. Most patients (13/17) had a proven or presumptive family history of primary immunodeficiency, compatible with the high percentage of consanguinity found in our cohort. The mean age at diagnosis was suboptimal, at almost 6 months (range 0–16), with the unconditioned group being diagnosed significantly earlier than the conditioned one (3.3 ± 2.3 vs. 7.1 ± 4.4 months, respectively; p value < 0.05). Immunological evaluation was positive for disease in all patients— absolute and relative lymphocyte deficiencies and undetectable TRECs. Thorough genetic testing managed to discover the disease-causing mutation in 15/17. A consequent of the consanguinity mentioned above, 14 patients had autosomal recessive mutations—11 with *RAG1/2*, 2 siblings with *ADA*, and 1 with a *JAK3* mutation. One patient suffered from X-linked SCID (common γ chain deficiency), and the culprit mutations of the remaining two evaded our tests. Table 1 summarizes immunological evaluation at diagnosis.

Transplantation

Patients were retrospectively classified into two groups based solely on the presence or absence of pre-transplantation conditioning: group 1 consisted of seven patients who did not receive

Table 1 Pre-HSCT workup of the patients, including lymphocyte subpopulations, cell-mediated, and humoral immunity

Patient	Flow cytometry analysis (lymphocytes in 10^6/L)						Cell-mediated immunity	Humoral immunity (mg/dL)		
	ALC	CD3	CD4	CD8	CD20	CD56	TREC (copies)	IgG	IgA	IgM
	3500–9000	1900–5900	1400–4300	500–1700	600–2600	160–950	> 400	230–1400	0–-80	0–-140
1	809	0	0	210	0	502	UD	260	UD	UD
2	873	0	0	245	0	585	UD	970	UD	UD
3	649	0	0	0	532	0	UD	79	UD	UD
4	89	65	25	2	0	1	UD	352	UD	UD
5	284	0	0	34	0	233	UD	859	UD	UD
6	106	23	13	18	2	17	UD	411	UD	UD
7	857	0	86	146	0	406	UD	161	UD	UD
8	2291	3668	1074	2594	45	447	8	808	UD	UD
9	1320	488	224	224	0	N/A	UD	869	UD	UD
10	5490	4612	2855	1757	44	494	5	433	80	UD
11	746	2671	2351	855	0	3847	UD	12.5	UD	110
12	3128	0	31	313	0	188	N/A	831	UD	UD
13	405	16	4	12	365	20	UD	146	UD	80
14	720	259	310	194	0	216	UD	1050	UD	UD
15	8721	6541	3576	3314	0	N/A	UD	435	UD	UD
16	14,807	4442	3109	4590	0	4294	UD	2340	72	26
17	658	202	134	183	96	519	UD	1400	91	1170

N/A not available, *UD* undetectable

conditioning (patients 1–7) and ten patients who did receive (patients 8–17). The choice whether to opt a conditioning or unconditioning protocol was based on SCID type, available donor, presence of Omenn syndrome or maternal engraftment, patient performance status, age, and future access to IVIG. Overall, the mean age at transplantation of the cohort was 252 days (range 61–657), a dissatisfactory fact, explained by the rather late age of diagnosis noted above. The entire unconditioned groups' donors were MRDs—mostly siblings, but also two mothers and a cousin. Five of seven grafts were from bone marrow, one from maternal peripheral blood, and one from the cord blood of a newly born sibling. The conditioned group varied much more—five haplo donors, two MUDs, and three patients who underwent conditioning even though an MRD was available—due to Omenn syndrome, old age, and advanced disease. In accordance with the higher donor age, more grafts (six of ten) were from peripheral blood, three from bone marrow, and one from cord blood.

Conditioning was done by a variety of regimens. As described, GVHD prophylaxis, when used, was based mainly on CSA. MMF and corticosteroids were added when GVHD was suspected or diagnosed. Acute and chronic GVHD appeared only in conditioned patients, all of which received adequate prophylaxis. Febrile episodes were also slightly more prevalent in this group. Table 2 summarizes the clinical and genetic features of the patients pre-HSCT. Characteristics of the HSCT procedures and their complications are depicted in Table 3. Despite physical and pharmacological measures, viral and/or bacterial infections complicated all patients post-HSCT. Quite uniquely to our cohort is the fact that 13/17 received the *Bacillus Calmette–Guérin* (BCG) vaccine postnatally, before their diagnosis. Consequentially, nine developed local or disseminated BCG infections ("BCGitis") and were treated effectively with anti-mycobacterial regimen which included rifampin, isoniazid, and ethambutol.

Immune reconstitution

Comparison of pre- vs. post-transplant lymphocyte subsets demonstrates quantitative improvement, with limited clinical correlation. Lymphopenia (defined as an ALC < 2500) was exhibited by 13/17 patients (76%) beforehand, and in 9/15 (60%) at end of follow-up, with conditioned patients less lymphopenic than unconditioned ones (p value < 0.05). CD3 cell was initially deficient (CD3 < 1000) in 12/17 (71%), a percentage later improving to 33% (5/15). A few factors confound pre-transplant T cell numbers—concurrent infections causing lymphocytosis, Omenn phenotype, and maternal engraftment. As of CD20 counts, all B− patients were deficient (CD20 < 250) before transplantation, improving later in 4 patients (9/13, 69%), again, all from the conditioned group (p value < 0.05).

Pre-transplant TREC levels were, by definition, undetectable or insignificant in all patients. Levels were serially quantified along the follow-up, showing gradual and steady increases. Out

Table 2 Clinical and genetic features of the patients

Patient	Age at diagnosis (months)	Gene defect	Mutation	Family history of SCID	Consangunity
1	5	RAG2	c.G104T, p.G35V	Yes	No
2	5	RAG2	c.G104T, p.G35V	Yes	Yes
3	1	JAK3	Unknown	Yes	Yes
4	5	ADA	c.A50C, p.H17P AND IVS9+1G>T	Yes	No
5	6	RAG1	Unknown	Yes	Yes
6	1	ADA	c.A50C, p.H17P AND IVS9+1G>T	Yes	Yes
7	2	RAG1	del.4bp, c.1407-TTGC	Yes	Yes
8	7	Unknown	Unknown	No	No
9	4	RAG2	c.G104T, p.G35V	Yes	Yes
10	6	RAG2	c.G471T, p.G157V	No	Yes
11	4	RAG2	G95V and E480X	No	No
12	3	RAG2	c.G471T, p.G157V	Yes	Yes
13	5	IL2RG	c.C241T, p.Q81X	Yes	No
14	16	RAG2	c.G218A; p.R73H	No	No
15	6	RAG1	del.4bp, c.1407-TTGC	Yes	Yes
16	6	RAG1	del.4bp, c.1407-TTGC	No	Yes
17	14	Unknown	Unknown	Yes	Yes

RAG 1/2 recombination activating gene 1/2, *ADA* adenosine deaminase, *JAK3* janus kinase 3, *IL2RG* interleukin-2 receptor subunit gamma

Table 3 Characteristics of the BMT procedures and its related complications

Patient	Age at HSCT (months)	Donor	Donor type	Sex	Graft	Conditioning	GVHD Prophylaxis	Acute GVHD	Chronic GVHD	Febrile episodes	Chimerism (donor %)	Follow-up (months)
1	8.8	Mother	MRD	F → F	BM	None	CSA	–	–	1	23	15
2	8.2	Cousin	MRD	M → F	BM	None	CSA	–	–	2	13	15
3	2	Sibling	MRD	F → M	CB	None	–	–	–	0	4	6
4	6.3	Sibling	MRD	M → M	BM	None	CSA	–	–	1	20	15
5	9	Sibling	MRD	M → M	BM	None	–	–	–	1	38	15
6	2.1	Sibling	MRD	F → M	BM	None	CSA	–	–	1	36	13
7	5	Mother	MRD	F → F	PB	None	CSA	–	–	1	68	5
8	9.1	Mother	HAPLO	F → F	PB	BU/CY/TT	MMF	–	–	0	100	14
9	6.4	Cousin	MRD	F → M	BM	TREO/FLU/TT	CSA	+	+	1	100	14
10	8.7	Mother	HAPLO	F → M	PB	FLU/TT/MEL/OKT3	MMF	+	–	4	66	12
11	5.9	Unrelated	MUD	N/A → M	CB	BU/CY/TT	CSA	+	–	1	100	12
12	5.9	Mother	HAPLO	F → M	PB	FLU/ATG	N/A	N/A	N/A	N/A	22	1
13	6.3	Mother	HAPLO	F → M	PB	FLU/TT/MEL/ATG/RTX	CSA, MMF	–	–	2	8	17
14	21.9	Unrelated	MUD	N/A → M	BM	TREO/FLU/ATG	CSA	+	+	4	100	13
15	8.7	Mother	HAPLO	F → M	PB	FLU/TT/MEL/ATG/RTX	MMF	–	–	1	5	10
16	11.3	Uncle	MRD	M → M	PB	TREO/FLU/CAMP	CSA	–	–	1	69	12
17	17.3	Sibling	MRD	M → M	BM	BU/FLU	CSA	–	–	1	100	10

ATG anti-thymocyte globulin, *BM* bone marrow, *BMT* hematopoietic stem cell transplantation, *BU* busulfan, *CAMP* campath, *CB* cord blood, *CSA* cyclosporin, *CY* cyclophosphamide, *FLU* fludarabine, *GVHD* graft vs. host disease, *MEL* melphalan, *MMF* mycophenolate mofetil, *MRD* matched related donor, *MUD* matched unrelated donor, *N/A* not available, *OKT3* orthoclone, *PB* peripheral blood, *RTX* rituximab, *TT* thiotepa, *TREO* treosulfan

of 15 live patients, 10 returned to what we consider normal TREC values (> 400 copies) by the study's end, without a statistically significant difference between groups. On average, conditioned patients demonstrated a faster incline in TREC levels compared with unconditioned ones and reached higher endpoint levels (1605 ± 1351.3 vs. 620.8 ± 990.9 copies). Yet, the unconditioned group showed an earlier presence of TRECs when compared during the first 4 months post-HSCT (25.6 ± 60.3 vs. 59.3 ± 78.6 copies), though trends soon decussated in the following quadrimester (283.8 ± 408.8 vs. 653.6 ± 946.2 copies). As anticipated, high TRECs had a positive predictive value concerning prognosis, though the opposite is less evident. Figure 1 demonstrates TREC dynamics along the follow-up.

Two factors influenced analyses of KRECs. First, KRECS were not part of the regular post-transplant workup, but rather conducted retrospectively on available specimens. Second, patients who initially had B+ SCIDs (#3 and #13) must be distinguished from the latter B− patients. Overall, the unconditioned cohort's KREC levels varied between undetectable to low (15.8 ± 33.1 copies), coinciding with their categorical dependency on IVIG infusion at the end of follow-up (7/7). In the conditioned group, KRECs were satisfactory in most patients (1439.3 ± 1511.4 copies, p value < 0.01). Two of eight relied on IVIG—patient #10 who indeed had unsatisfactory KRECs and patient #15 whose specimens were unavailable for testing. Conditioned patients had a higher chance of being IVIG independent at the end of their follow-up (p value < 0.01). Table 4 summarizes patients' basic cell-mediated and humoral immune reconstitution.

Long term follow-up and clinical outcomes

Mean follow-up for the entire cohort was 355 days from HSCT, ranging from 18 to 511 days. Overall survival was excellent (88%). Although the aim of this study was to follow patients for the year after their HSCT, 14 of them are still observed periodically at the PID and/or the hemato-oncology clinics at our institution and are alive and well, some over 9 years past their procedure. Patients #7 and #12 unfortunately died soon after their HSCTs—the former from septic shock 6 months post her transplant and the latter from early HSCT complications, just days after his procedure. Patient #3 was lost to follow-up at our hospital but is known to be in satisfactory condition.

As expected, the engraftment as portrayed by lymphocyte microsatellites and FISH followed opposite trends between groups (Fig. 2). Unconditioned patients' engraftment began from near 0% and raised steadily, eventually reaching a mean of 30% (range 4–68%) at the end of follow-up. Conditioned patients began from full engraftment (kept by 5/10) and lost by the remaining who declined to values averaging 34% (range 5–69%). Engraftment chimerism did not correlate with clinical outcomes, corroborating the notion that full engraftment is not necessarily an important goal. It is imperative to note that due to differences in the follow-up technique between patients, we

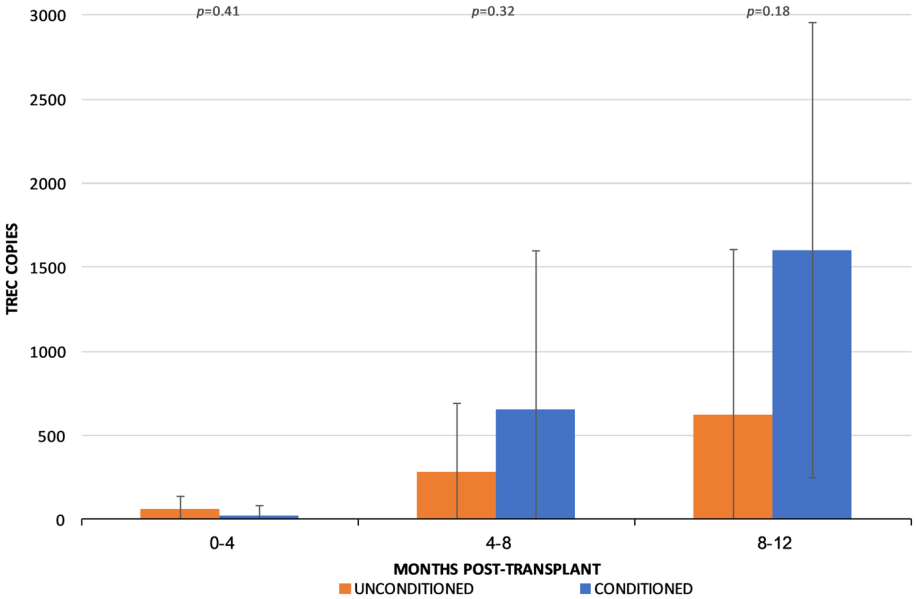

Fig. 1 TREC levels in the conditioned group and the unconditioned group along three quadrimesters in the post-transplant year. TRECs were serially checked along the follow-up. The unconditioned group showed an earlier presence of TRECs immediately after transplantation (59.3 ± 78.6 vs. 25.6 ± 60.3 copies), though trends soon decussated in the following quadrimester (283.8 ± 408.8 vs. 653.6 ± 946.2 copies), eventually reaching higher endpoint levels in the conditioned group (620.8 ± 990.9 vs. 1605 ± 1351.3 copies). High TRECs had a positive predictive value concerning prognosis, though the opposite is less evident

used FISH and microsatellite percentages interchangeably, acknowledging that only subtle differences occur between their results [12]. Table 5 summarizes and compares various parameters between the groups.

Table 4 Cell-mediated and humoral immune reconstitution after HSCT, including outcome and IVIG dependency

Patient	Months post-HSCT	Flow cytometry analysis (lymphocytes in 10^6/L)						Cell-mediated immunity	Humoral immunity (mg/dL)					Outcome
		ALC	CD3	CD4	CD8	CD20	CD56	TREC (copies)	IVIG	IgG	IgA	IgM	KREC (copies)	
1	15	1855	1484	798	649	0	186	> 400	+	265	56	28	0	Alive
2	16	2184	896	721	306	0	764	22	+	627	UD	UD	0	Alive
3	6	1565	736	595	250	720	0	> 400	–	618	UD	43	25,353	Alive
4	15	1034	941	248	414	52	217	37	+	784	UD	38	175	Alive
5	15	1893	1628	720	890	0	284	> 400	+	1040	UD	28	N/A	Alive
6	14	1449	1275	391	797	23	290	38	+	1450	230	50	4	Alive
7	5	N/A	N/A	N/A	N/A	N/A	N/A	94	+	931	54	27	N/A	Deceased
8	14	7450	4768	2384	1639	1639	522	> 400	–	1010	102	156	1590	Alive
9	14	5084	3711	2695	1118	763	203	> 400	–	819	60	79	N/A	Alive
10	12	1584	1220	649	523	63	79	> 400	+	371	UD	20	526	Alive
11	12	4802	3169	2017	912	1345	288	> 400	–	556	UD	63	3812	Alive
12	1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Deceased
13	17	5295	4501	2012	2383	635	265	> 400	–	1040	UD	74	1295	Alive
14	13	5055	3690	1668	1971	708	354	> 400	–	783	32	23	2550	Alive
15	10	713	385	349	78	0	214	30	+	385	UD	UD	N/A	Alive
16	12	4526	2852	1086	1448	498	769	> 400	–	984	59	32	79	Alive
17	10	1681	992	538	269	488	84	231	–	1070	67	158	79	Alive

ALC absolute lymphocyte count, *BMT* bone marrow transplant, *IVIG* intravenous immunoglobulin, *KREC* kappa-deleting recombination excision circles, *N/A* not available, *TREC* T cell recombination excision circles

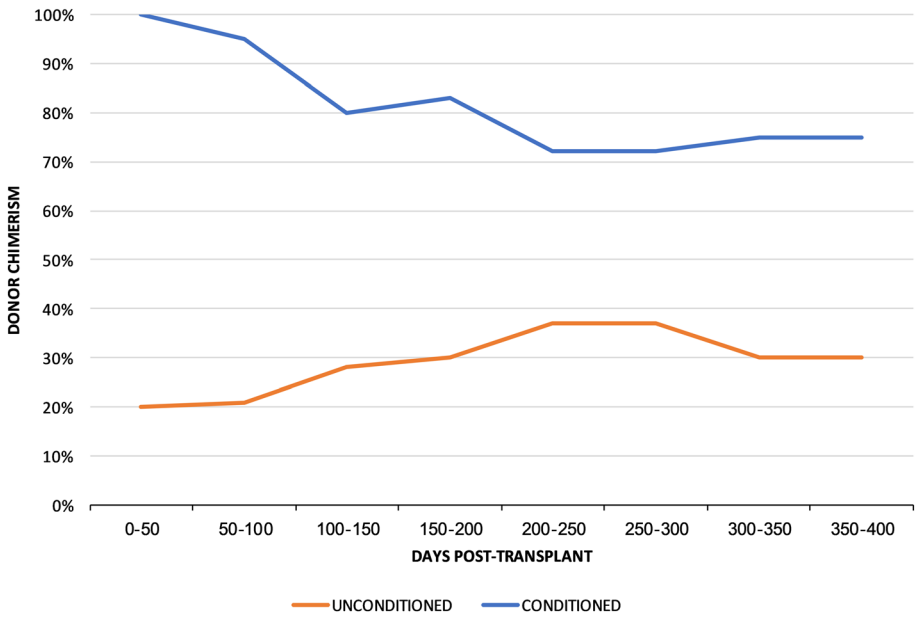

Fig. 2 Average donor lymphocyte chimerism in the post-HSCT year. Donor lymphocyte engraftment was assessed interchangeably by microsatellite or FISH studies. Averages of each group are depicted above. The conditioned group began from full engraftment (kept by half), slowly declining to an average of 75% (range 5–100%). The unconditioned group began from near 0%, slowly inclining to average 30% (range 4–68%). Engraftment did not correlate with clinical outcomes

Discussion

SCID, the most severe type of congenital immunodeficiency, is curable by HSCT. The use of conditioning before HSCT for SCID and its effect on long-term outcome remains controversial and is a subject which calls for continuing research [13, 14]. Herein, we described 17 patients with SCID who underwent HSCT with or without conditioning at our center.

Overall 1-year survival rate in our cohort, regardless of the presence or absence of pre-transplantation conditioning, was excellent (88%). This is similar to results obtained from several large single or multicenter studies comparing survival in SCID after HSCT, probably attributed to our high percent of MRDs (10/17 patients). The use of matched related and unrelated donors (including cord blood) whenever possible and limiting the use of mismatched haploidentical donors are well-accepted approaches now [15]. Our results when using mismatched haploidentical donors were satisfactory, and only one such recipient deceased [4, 16].

Donor compatibility is a factor of paramount importance, but still second to prompt diagnosis and treatment. In their multicenter study, Pai et al. have shown that survival rates are similar among infants who received transplants at 3.5 months of age or younger (94%) and among older infants without prior infection (90%) or with a resolved infection (82%), regardless of donor type [17]. The late diagnosis and HSCT timing (averaging 5.9 and 10.2 months, respectively) of our patients is an issue that demands introspection and action. In the era of newborn screening, diagnosis must precede clinical manifestations and transplant should occur at the recommended age of 3.5 months [18]. The whole group was diagnosed and treated before 2016, when the Israeli national newborn screening program began to implement TREC quantification as a method for early recognition of T cell–deficient SCIDs. Reports from other countries, along with preliminary data from Israel, make it safe to assume that the screening program will substantially shorten the intervals from birth to diagnosis and diagnosis to transplant [19, 20]. Yet, a large portion of our patients come from the Gaza Strip and the West Bank, a fact that has significant medical impacts. First, there is no newborn screening there. Second, Palestinian neonates receive the BCG vaccine at birth, known to cause a very high complication rate in patients with SCID. Therefore, until the establishment of newborn screening for SCID there, and in the absence of a safer vaccine, this routine vaccination should be postponed, particularly when family history indicates former PIDs [21].

Another aspect in which our cohort differs from traditional cohorts is genetically. As noted, at least 82% of our patients had autosomal recessive SCID variants, with the T−B−NK+ phenotype comprising the majority and the *RAG1/2* genotype being the largest subset. This is quite consistent with data from regional and nationwide studies, reflecting both the high level of consanguinity in our patient population (autosomal recessive > X-linked), and the "founder effect," influencing which of the autosomal recessive mutations is the prevalent one (e.g., *RAG1/2* > *ADA*) [22, 23]. Several conclusions concerning treatment and prognosis may be drawn from this fact. The first involves finding an MRD—most patients with SCID must settle for an alternative donor source, as only about 20% have an available one. Contrarily, in highly consanguineous populations, there is a higher chance of finding a next of kin who is an MRD [24]. Indeed, almost 60% of our patients received HSCTs from MRDs. The second regards B cell engraftment,

Table 5 Comparison of various immunological and clinical factors between the conditioned and the unconditioned group

Criteria	Unconditioned			Conditioned			*p* value	Total		
	Number	Sum	Percent	Number	Sum	Percent		Number	Sum	Percent
Pre-BMT										
Total patients	7	–	–	10	–	–	–	17	–	–
Age at diagnosis (months)	3.3	–	–	7.1	–	–	< 0.05	5.5	–	–
Age at HSCT (months)	5.9	–	–	10.2	–	–	NS	8.4	–	–
B cell negative	6	7	86%	9	10	90%	–	15	17	88%
ALC < 2500	7	7	100%	6	10	60%	NS	13	17	76%
CD3 < 1000	7	7	100%	5	10	50%	< 0.05	12	17	71%
CD20 < 250[a]	6	6	100%	9	9	100%	NS	15	15	100%
TREC < 400	0	7	0%	0	9	0%	NS	0	16	0%
Mode of transplant										
Donor type (MRD/MMRD/MUD)	7	0	0	3	5	2	–	10	5	2
Graft (PB/CB/BM)	1	1	5	6	1	3	–	7	2	8
GVHD prophylaxis	5	7	71%	9	9	100%	–	14	16	88%
End of follow-up										
Acute GVHD	0	7	0%	4	9	44%	NS	4	16	25%
Chronic GVHD	0	7	0%	2	9	22%	NS	2	16	13%
Febrile episodes[b]	6	7	86%	8	9	89%	NS	14	16	88%
BCGitis[c]	5	5	100%	4	6	67%	NS	9	11	82%
ALC < 2500	6	6	100%	3	9	33%	< 0.05	9	15	60%
CD3 < 1000	3	6	50%	2	9	22%	NS	5	15	33%
CD20 < 250[a]	5	5	100%	4	8	50%	< 0.05	9	13	69%
TREC < 400	3	6	50%	2	9	22%	NS	5	15	33%
KRECs[a] (copies)	15.8	5	–	1439.3	6	–	< 0.01	792.3	11	–
IVIG dependency[a]	6	6	100%	2	8	25%	< 0.01	8	15	53%
Chimerism	7	–	29%	10	–	67%	NS	17	–	51%
Alive and well	6	7	86%	9	10	90%	NS	15	17	88%

ALC absolute lymphocyte count, *BCG Bacillus Calmette–Guérin* (vaccine), *HSCT* hematopoietic stem cell transplantation, *CB* cord blood, *GVHD* graft vs. host disease, *IVIG* intravenous immunoglobulin, *KREC* kappa-deleting recombination excision circles, *MMRD* mismatched related donor, *MRD* matched related donor, *MUD* matched unrelated donor, *NS* not significant, *PB* peripheral blood, *TREC* T cell recombination excision circles

[a] B cell–negative patients only

[b] Patients with one or more febrile episodes, excluding patient #12 who died soon after transplant

[c] Excluding patients who did not receive the BCG vaccine

as our cohort was relatively abundant in B cell–deficient patients. The molecular type of SCID is the main factor that influences B cell reconstitution. Host B cell function may normalize even without conditioning, particularly in B+ patients. While conditioning does not guarantee B cell engraftment and function, it does significantly improve the chance that it will occur [25, 26]. All our B− unconditioned patients (six of six) remained dependent on IVIG at the end of follow-up, despite receiving transplants from MRDs. Sixty-seven percent (four of six) of our B− conditioned patients regained enough B cell function to stop routine IVIG infusions. Data from a study by Heimall et al., where 47% of conditioned vs. 14% of unconditioned were off IVIG at 1-year post-transplant, also supports this concept [27]. To our patients, this brings forth another consideration when contemplating which transplantation regimen to conduct—accessibility to centers that provide IVIG therapy. In patients whose compliance and access to IVIG is expected to be limited, the tension between better B cell engraftment and a riskier transplantation may be shifted towards the latter.

Appropriate immune reconstitution is a major contributor to long-term quality of life in patients with SCID after HSCT. A myriad of quantitative and qualitative tests have been suggested for the follow-up of transplantees, and no specific battery has been proven superior. We, and others, have successfully used TRECs and KRECs as markers for T and B cell immune reconstitution and as predictors for post-transplant morbidity and mortality [5, 28]. Here,

TRECs were an excellent mean of diagnosis and follow-up. Attaining normal levels (> 400) was concordant with a favorable outcome in all cases. The opposite may not be concluded, as several well-recovering patients had suboptimal TREC levels at the end of their follow-up. In the first quadrimester, unconditioned recipients exhibited higher TREC levels than conditioned ones. From that point until the end of follow-up, the conditioned group's TRECs were superior. KRECs were not followed in a sequential manner; therefore, their predictive value was not assessed. Yet, the correlation between sufficient KREC levels and independency from IVIG in our study, and their proved role as heralds of B cell function, may promote their use as early markers for beginning IVIG weaning [5].

Engraftment chimerism trends were opposite between groups. Patients after chemoablation began from full chimerism, some maintaining it and some transiting into mixed chimerism. On the other hand, unconditioned patients began from null, developing engraftment with time. The exact desired donor engraftment is not defined, but both groups managed to average > 10% chimerism (deemed sufficient by some sources [29]), and most importantly were free of significant infections. We assume that lineage-specific chimerism is much more important than the general chimerism, as depicted by the fact that some patients with low total chimerism eventually displayed sufficient TRECs, still resulting in a better clinical outcome. In recent years, advanced assays (such as TRECs, KRECs, TCR repertoire) brought the advantage of quantitative and qualitative evaluation of immune recuperation and lessened the role of chimerism studies in long-term follow-up [30].

To summarize, we studied a cohort of severely immunodeficient patients who underwent life-saving medical procedures. Though differing in laboratorial and clinical data, we showed excellent outcomes with both treatment methodologies and debated the pros and cons of their application. The small cohort, together with the differences in genotypes, ages, donor types, and procedures, limitations of the study, withholding deduction of significant statistical conclusions. Nevertheless, the study contributes to the body of knowledge concerning the treatment of SCID and to the support of TRECs and KRECs as surrogates of immune reconstitution and tailored therapy. Concerning the dilemmas featured by most of our patients—consanguinity, BCGitis, accessibility, etc.—the study delineates important considerations. Furthermore, it reiterates the importance of newborn screening and obtaining family history as secondary preventive measures—granting early diagnosis as well as averting opportunistic and vaccine-related infections.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Human and animal rights statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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