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ORIGINAL ARTICLE

Clinical Features and HSCT Outcome for SCID in Turkey

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Abstract

Severe combined immunodeficiency (SCID) is the most serious PID, characterized by T cell lymphopenia and lack of antigen-specific T cell and B cell immune responses, inevitably leading to death within the first year of life if hematopoietic stem cell transplantation (HSCT) is not performed.

Purpose and Methods

Since SCID is a common type of PID with an estimated incidence of 1/10.000 in Turkey, a retrospective analysis of HSCT characteristics, survival, immune recovery, and the major clinical features of SCID prior to HSCT is the aim of this multi-transplant center-based analysis.

Results

A total of 234 SCID patients transplanted between the years 1994 and 2014 were included in the study. Median age at diagnosis was 5 months, at transplantation, 7 months, B− phenotype and RAGs were the most common defects among others. Immune phenotype did not seem to have an effect on survival rate ($p > 0.05$), Immunoglobulin (Ig) requirement following HSCT did not differ between B+ and B− phenotypes ($p > 0.05$). Overall survival rate was 65.7% over a period of 20 years. It increased from 54% (1994–2004) to 69% ($p = 0.052$) during the last 10 years (2005–2014). Ten-year survival after HSCT has improved over time although the difference was not significant. Infection at the time of transplantation ($p = 0.006$), mismatched related donor (MMRD) (haploidentical parents), and matched unrelated donor (MUD) donor transplants $p < 0.001$ were the most important factors, significantly affecting the outcome.

Conclusions

This is the first multicenter study with the largest data obtained from transplanted SCID patients in Turkey. Early diagnosis with newborn screening (NBS) together with emerging referrals, treatment by transplantation centers, and specialized teams are mandatory in countries with high parental consanguinity such as Turkey.

Keywords Severe combined immune deficiency (SCID) · Hematopoietic stem cell transplantation (HSCT) · clinical features · outcome

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Introduction

Severe combined immunodeficiency (SCID) is the most serious PID, characterized by T cell lymphocytopenia and/or lack of antigen-specific T cell and B cell immune responses [1]. These patients are highly susceptible to infections especially opportunistic ones beginning within months after birth. It is a genetically heterogeneous disorder of infancy, normally leading to death within the first year of life if hematopoietic stem cell transplantation (HSCT) is not performed [1, 2]. SCID is a pediatric emergency and early diagnosis via newborn screening (NBS) is a lifesaving approach.

There are autosomal recessive and X-linked defects leading to SCID. Although the X-linked form is more prevalent in western countries, autosomal recessive forms are more common in Turkey due to high parental consanguinity rate [3]. A regional pilot study determined SCID incidence as 1/10.000 in Turkey [4]. Here, we present the HSCT characteristics, immune recovery, survival data, and the major clinical features of SCID prior to HSCT in 234 patients from multi-transplant centers of Turkey.

Methods

Patient Details

All Pediatric Transplantation centers in Turkey were asked to participate in this study. Data have been collected retrospectively from 234 transplanted infants out of 296 with SCID from eight different centers over a period of 20 years (1994–2014). All patients fulfilling the SCID criteria according to the latest published classification are included [5].

The Centers contributing to this study and number of cases submitted by each center were as follows: Ankara, Hacettepe University ($n = 94$), Ankara University ($n = 48$), Istanbul, Göztepe MP ($n = 25$), Izmir, Ege University ($n = 20$), Antalya MP ($n = 20$), Antalya, Akdeniz University ($n = 16$), Samsun, Ondokuz Mayıs University ($n = 9$), and Ankara, GATA University ($n = 2$). Molecular genetic defect was identified in 99 patients (42.3%). The age of the patients on admission, at HSCT, and at the last follow-up, their gender, parental consanguinity, BCG vaccination prior to diagnosis and immune phenotype of SCID were recorded. The presence of infections including infected organs/systems, type of infections, and growth and organ failures were evaluated by reviewing patient's files.

HSCT Characteristics

The HSCT characteristics including donor and HSCT source, conditioning regimen, graft versus host disease (GvHD) prophylaxis; transplant-related complications such as acute and chronic GvHD, venooclussive disease (VOD), BCG activation, and retransplants were also evaluated.

Immune Reconstitution

T and B cell recovery at 2–5 years after HSCT and related data were obtained. T cell recovery was defined as CD3+ ($> 50\%/1000/mm^3$) and CD4+ ($> 25\%/> 500/mm^3$) enumeration and activation/proliferation response towards PHA. B cell reconstitution was defined as CD19+ or CD20+ B cell ($> 10\%/> 400/mm^3$) enumeration, serum IgA level, and IVIG independence.

Statistical Tools

Various statistical methods were used for data evaluation.

The differences between groups were compared by using Chi-Square or Fisher's Exact test, where appropriate. A p value of less than 0.05 was considered statistically significant. Probabilities of survival after transplantation were calculated with Kaplan-Meier estimator. Multivariate Cox regression models were built to examine the risk factors for transplantation outcome.

Results

The Major Clinical Features of SCID Prior to HSCT

Patients, 145 (62%) male and 89 (38%) female with a median age of 5 months (ranged between 0.25 and 176 months) at the time of diagnosis were transplanted. Parental consanguinity was identified in 76.3% of 224 cases of whom 133 (59.4%) were first cousins. 72% had received BCG vaccination prior to diagnosis. INAH and Rifampycin prophylaxis were given to all vaccinated cases. BCGitis was reported in three patients at diagnosis and in five at time of HSCT. Growth and organ failures, mainly lung dysfunction, were presented in more than half of the cases (51%). B− phenotype was prominent with few ADA and RD patients. The demographic and clinical characteristics of 234 patients are given in Table 1.

Genetic cause of SCID was identified in 42.3% of the infants, predominantly AR mutations in RAG1 (9.8%), RAG2 (5.6%), Artemis (5.6%), and JAK3 (6.8). Out of 234 patients, 11 with T-B− NK- immune phenotype and five other with neutropenia and thrombocytopenia in addition to lymphopenia were diagnosed as ADA deficiency and reticular dysgenesis respectively, without a confirmed molecular analysis. The genetic characteristics of patients are given in Fig. 1. Infections were documented in 85% of

Table 1 Demographic and clinical characteristics of patients

Age at diagnosis (mos) [range (median)] ($n = 234$)	0.25–176 (5)
Gender (female/male) (n = 234)	89 (38%)/145 (62%)
Parental consanguinity ($n = 224$)	171 (76.3%)
1st degree	133 (59.4%)
2nd degree	14 (6.2%)
3rd degree	24 (10.7%)
BCG vaccination prior to diagnosis ($n = 229$)	164 (72%)
Growth failure ($n = 232$)	130 (56%)
Organ dysfunction/failure ($n = 232$)	
None	98 (42%)
Lung	119 (51%)
Liver	5 (2.2%)
Other	10 (4.3%)
Immune phenotype ($n = 232$)	
T-B+	97 (42%)
T-B−	119 (51%)
ADA	11 (4.7%)
RD	5 (2.2%)

mos months, *ADA* adenosine deaminase deficiency, *RD* reticular dysgenesis

the patients at diagnosis and 70% remained infected at the time of transplantation. Lung infections and persistent CMV antigenemia were the major problems (Table 2).

HSCT Characteristics of SCID

The median age was 7 months (ranged between 0.6 and 176 months) at transplantation. Almost half of the cases had either a matched sibling or a family donor, while 81 and 39 children received haplo (mismatch related donor-MMRD) and matched unrelated donor (MUD) transplants, respectively. Bone marrow (BM) was the leading source of stem cells followed by mobilized peripheral blood (MPB). 61.8% received unconditioned transplants. Myeloablative conditioning (MAC) was used in 64 patients (27.5%) and reduced intensity conditioning (RIC) was given to 15 (6.4%). Busulfan 8–16 mg/kg and Cyclopospamide 200 mg/kg or Busulfan 8–16 mg/kg and Fludarabine 160 mg/m^2 were used as MAC conditioning agents. RIC conditioning agents were: Fludarabine 150 mg/kg and Treosulfan 36 mg/m^2 with or without ATG 20–40 mg/kg (ATG added in MUD donor setting). Fludarabine 150 mg/kg, Cyclopospamide 20 mg/kg, and ATG 20 mg/kg were used in some selected patients with Cernunnos defect. More than half were given CsA. CD34 selection was performed mainly for haplos. Transplantation complications are seen in Table 3. 10% of the patients developed severe aGvHD (Grades 3–4) and 11% chronic GvHD. VOD occurred in 13 patients. BCG activation following engraftment was an important finding seen in one out of four patients (25%). Among a total of 24 (10.25%) retransplants, 18 received a second transplant while six children received only a boost (2.5%). Autoimmunity developed in 9% ($n =$

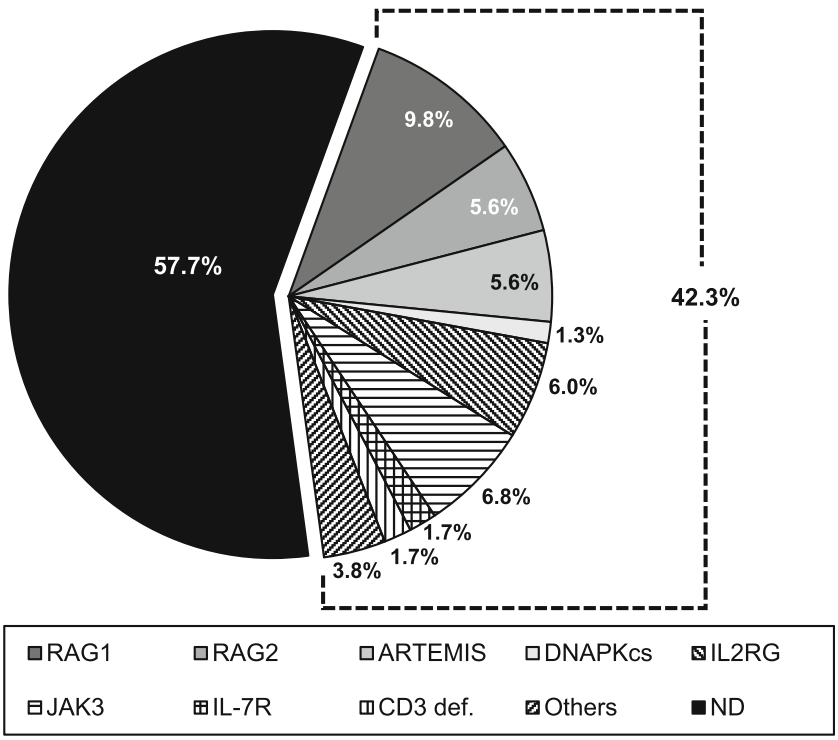


Fig. 1 The genetic characteristics of SCID patients

Table 2 Infections at diagnosis and at the time of transplantation

	At diagnosis ($n = 231$) (n/%)	At time of transplantation ($n = 233$) (n/%)
Yes	196/85	163/70
No	32/14	70/30
Infected organ/system		
Lung	69/35	35/21.5
GIS	15/7.7	4/2.5
Other	7/3.6	12/7.4
Multiple organs	66/34	23/14
Type of infection		
Bacterial	17/7.7	17/7.4
Respiratory viral (RSV, Rhino, PI)	4/1.9	11/4.4
BCGitis	3/1.4	5/2.2
CMV	36/16	31/13.5
Adenovirus	1/0.05	1/0.04
Candidiasis (local)	30/15.3	84/51.5
Undefined	135/69	98/60

PI parainfluenza

20). Mostly autoimmune hemolytic anemia and thyroiditis (3.6% in each) were detected in these patients.

Immune Recovery

T and B cell immunity was evaluated at 1, 2, and 5 years after transplantation by T and B cell enumeration and functional studies in 146 patients. T cell recovery was adequately achieved in almost 75% of the patents during the first 5 years following transplantation. B cell reconstitution remained at 50% (Fig. 2). Nevertheless, a significant difference ($p < 0.001$) was detected in B cell engraftment in patients with T-B+ phenotype (65.3%) and T-B− (34.7%). Serum IgA level ($n = 167$) was found to be normal in 85 patients (50.9%) whereas in 82, it appeared to be low compared to Turkish age-matched healthy children, without any positive contribution to survival ($p < 0.064$). The outcome found to be significantly better in patients whose T cells demonstrated normal response towards PHA ($p < 0.001$).

Survival and Outcome

After transplantation, 153 patients survived, 80 died (34.5%) mostly because of infections ($n = 52$, 68%) and one was lost to follow-up. Overall survival rate was 65.7% over a 20 years period. It was 54% for the first 10 years (1994–2004), increasing to 69% for the second 10 years (2005–2014). Ten-year survival after HSCT has improved over time although the difference was not significant ($p = 0.244$). Infections were the common cause of death followed by GvHD ($n = 9$, 12%). The survival rates in relation to donor types were 85.7% for MSD, 70.3% for MRD while 59% for MUD and 47.5 for haplos (MMRD). So, the outcome is poor in MUD ($p < 0.001$), MMRD ($p < 0.001$), and even in MRD ($p < 0.015$) compared to MSD and in unconditioned MMRD compared to MRD ($p < 0.041$) transplants (Fig. 3). Infection at the time of transplantation ($p = 0.006$), MMRD (haplo) and MUD donor transplants $p < 0.001$ were the most important factors significantly affecting the outcome (Table 4).

Discussion

It is obvious that the incidence of SCID is excessive in countries with a high rate of consanguinity. In fact, the rate of consanguineous marriages in Turkey is 23.2%, the eastern part of the country having much higher rate [6]. Therefore, B negative phenotype, especially RAGs were the most common defects among others in Turkey as shown before by Sanal and Tezcan [3].

The period between diagnosis and treatment (delay in treatment) is extremely important as the outcome differs significantly according to the age at HSCT [2]. Buckley at al. initially reported this significant feature almost two decades ago in 91 SCID patients. It was shown that 21 survived (95%) among 22 infants transplanted before 3.5 months old age, while 51 of 67 (76%) transplanted at or after 3.5 months were alive ($p < 0.088$) [7].

According to the latest data obtained from 240 patients with SCID in USA, an overall survival rate of 74% was achieved at 5 years. The survival rate was highest, reaching 94% in infants who received transplants at 3.5 months of age

Table 3 HSCT related complications

	n (%)
aGvHD ($n = 234$)	
Grade 1–2	76 (32.5)
Grade 3–4	23 (10)
cGvHD (2 yrs) ($n = 195$)	
Limited	16 (8)
Disseminated	6 (3)
VOD ($n = 228$)	13 (6)
BCG activation ($n = 218$)	
Local	39 (18)
Disseminated	8 (4)
Re transplants	24 (10.25)
Boost	6 (2.5)
Second transplant	18 (7.7)
Autoimmunity	20 (9)

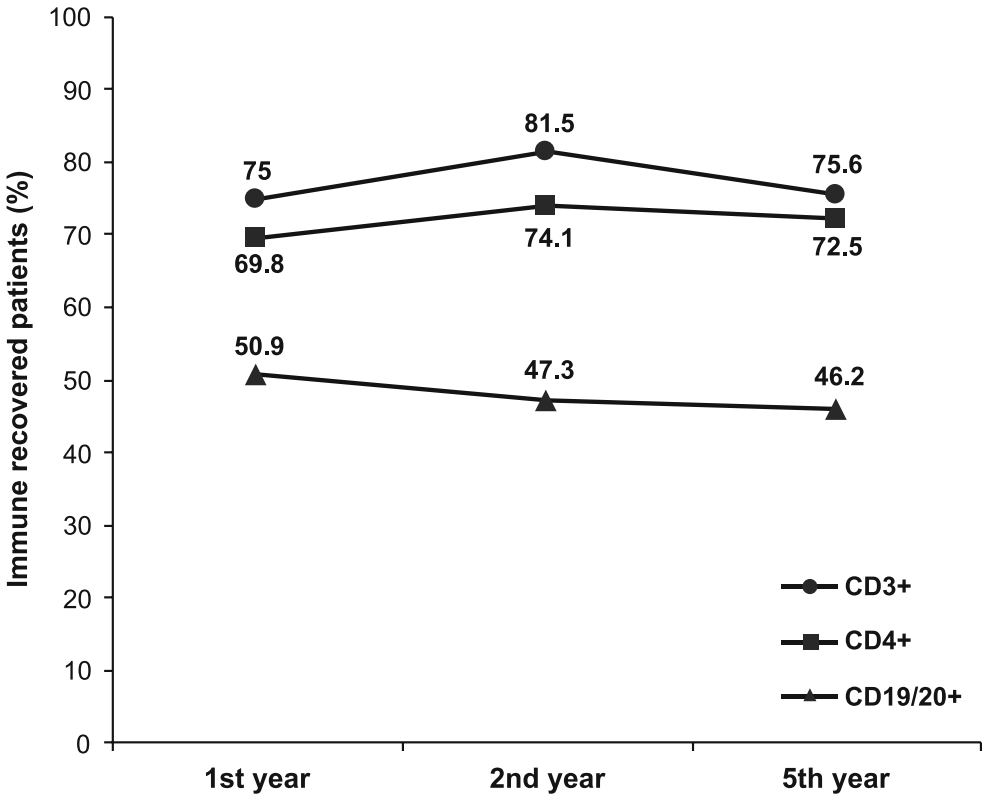


Fig. 2 T and B cell immune recovery at 1, 2, and 5 years after the transplantation

or younger and free from infections. However, the outcome deteriorated with active infections and increase in age. Moreover, the age at transplantation (less than 3.5 months), asymptomatic and free of infections with CD3+ T cell recovery were pointed out as the most significant factors affecting the outcome [8].

In the present study, the median age at diagnosis and at transplantation was 5 months and 7 months, respectively. The median age at diagnosis and at transplantation was 138.5 days and 180 days in the US cohort. The overall survival rate increases as the age at HSCT decreases [8, 9]. Nevertheless, the median age at diagnosis was quite earlier (2.15 months) in DiNardo et al.'s report with a much better overall survival achieved in few ($n = 25$) patients [9].

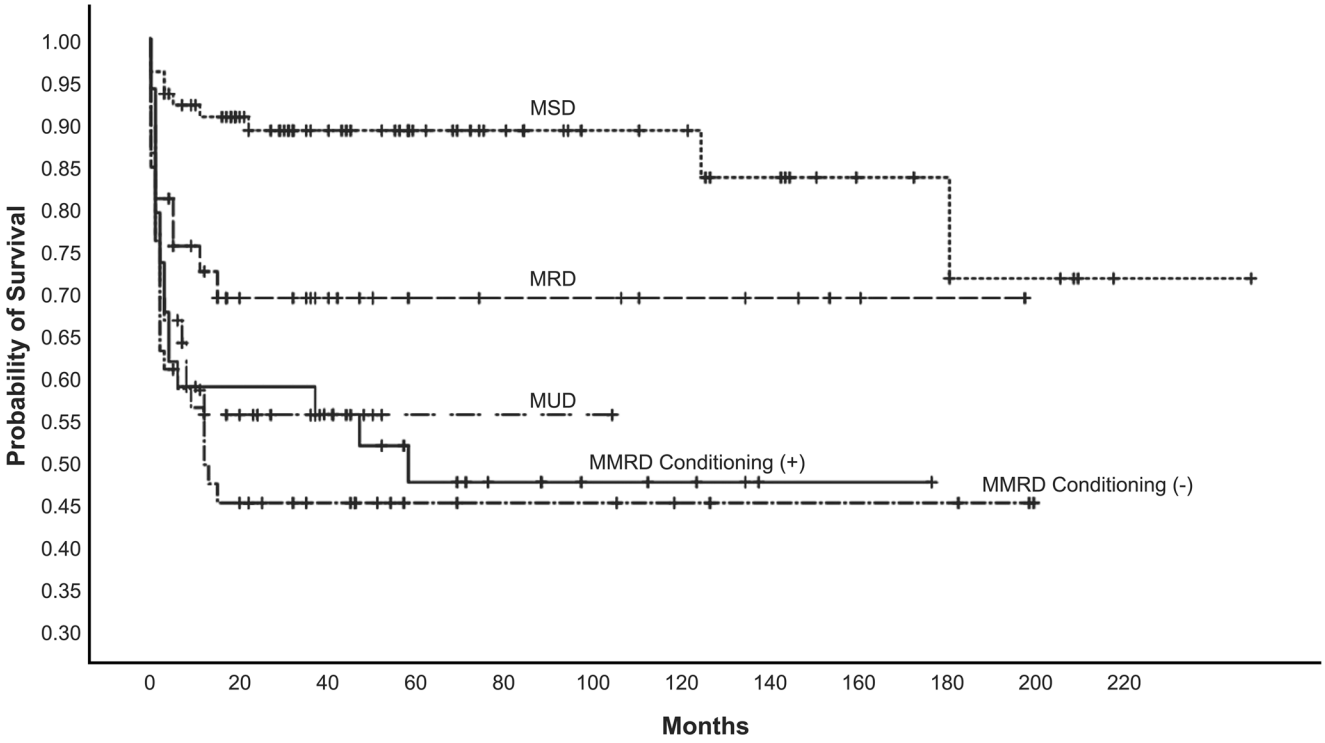
Pre-existing lung and liver failures; infections such as sepsis and meningitis; malnutrition were addressed as the factors associated with a worse outcome by Gennery et al. [10]. In our cohort, organ failures were detected in 58% of the patients.

Infections at the time of transplantation were shown to be the most important factor affecting the outcome, causing deaths. In our cohort, infectious episodes were the prominent cause of death (68% among all deceased patients). Being free from active infection at the time of allogeneic HSCT is an important predictor of survival [10]. Pai et al. reported that 71% of the patients have a documented infection prior to HSCT. It is 70% in the present study.

A mild aGvHD was identified in 76 (32.5%) patients, while 23 (10%) were severe cases. Chronic GvHD and autoimmunity were reported as 11–15% [8, 11] and 10–12% [11, 12] respectively in different studies. In the present study, mostly limited forms of cGvHD were detected in 11% of the cases whereas autoimmunity was recorded in 9% ($n = 20$). VOD occurred and was treated successfully in 13 patients.

BCG is a part of standard vaccination for infants to overcome severe tuberculosis in developing countries. However, it can cause BCG disease in various PIDs, especially in SCID. BCG complications were documented in 45% SCID cases, following a routine vaccination at birth by Yeganeh et al. [13]. In this study, BCGitis and disseminated disease occurred in 18% ($n = 39$) and 4% ($n = 8$) among 218 cases following engraftment. BCGitis is an inflammatory process, it mostly flares up during the early engraftment period.

The average rate of survival is > 70% at 3 years following transplantation [2, 14]. The overall survival (OS) rate of patients at 5 years was reported to be 74% by Pai et al. [8]. In our study, the OS rate was found to be 65.7% over a period of 20 years. It increased from 54 (1994–2004) to 69% ($p = 0.052$), from the first decade to the second one (2005–2014). 10-year survival in our patients after HSCT has improved over time although the difference was not significant ($p = 0.244$) (Fig. 4). Awareness, leading to early diagnosis and referrals, refinement in the HSCT techniques, and patient care are probably the most determining factors contributing to overall improvement in Turkey.



"p values" for Pairwise Comparisons of Expected Mean survival times.

	MSD	MRD	MUD	MMRD Conditioning (+)	MMRD Conditioning(-)
MSD		**0.015**	**<0.001**	**<0.001**	**<0.001**
MRD	**0.015**		0.237	0.160	**0.041**
MUD	**<0.001**	0.237		0.992	0.422
MMRD Conditioning (+)	**<0.001**	0.160	0.992		0.531
MMRD Conditioning (-)	**<0.001**	**0.041**	0.422	0.531	

Fig. 3 The survival in SCID in relation to donor types

In the study of Gennery et al., 10-year survival is significantly better in patients with B+ SCID. B− phenotype on the other hand, is leading to a poor outcome [10]. However, in the present study, immune phenotype did not seem to have an effect on the survival rate ($p > 0.05$), moreover, immunoglobulin (Ig) requirement following HSCT did not differ between B+ vs. B− phenotypes ($p > 0.05$). On the other hand, in our study, B cell numbers and serum IgA levels at 1, 2, and 5 years following HSCT were affected by B− phenotype ($p < 0.001$). So, the reason behind low B cell recovery following HSCT could be attributed to the predominance of T-B− SCID phenotype and transplant procedure (unconditioned).

Table 4 Outcome and contributing factors

Variables	HR[a]	95% CI[a]		P[a]
Infection at transplantation	2.281	1.266	4.111	0.006
MSD (reference category)				
MRD	2.168	0.901	5.218	0.084
MMRD	4.696	2.336	9.440	< 0.001
MUD	4.240	1.885	9.535	< 0.001

[a] Multivariate Cox regression model

In Pai et al.'s study, 70% of the patients [8] and 80% of the patients in the present study had CD3+ T cell counts exceeding 1000/mm^3. T cell recovery was adequately achieved in almost 75% of the patents during the 5 years following the transplantation. B cell reconstitution remained at 50%. Serum IgA levels were documented as normal in 50.9% of the patients after 2–5 years of follow-up and free from IVIG therapy. Response to PHA during 2–5 years following the transplantation was the only significant factor contributing to *t* survival. 96.7% of the patients with a normal response to PHA were alive while none survived with abnormal PHA response ($p < 0.001$).

Recently, a retrospective analysis of 662 SCID patients transplanted between 1982 and 2012 in 33 North American

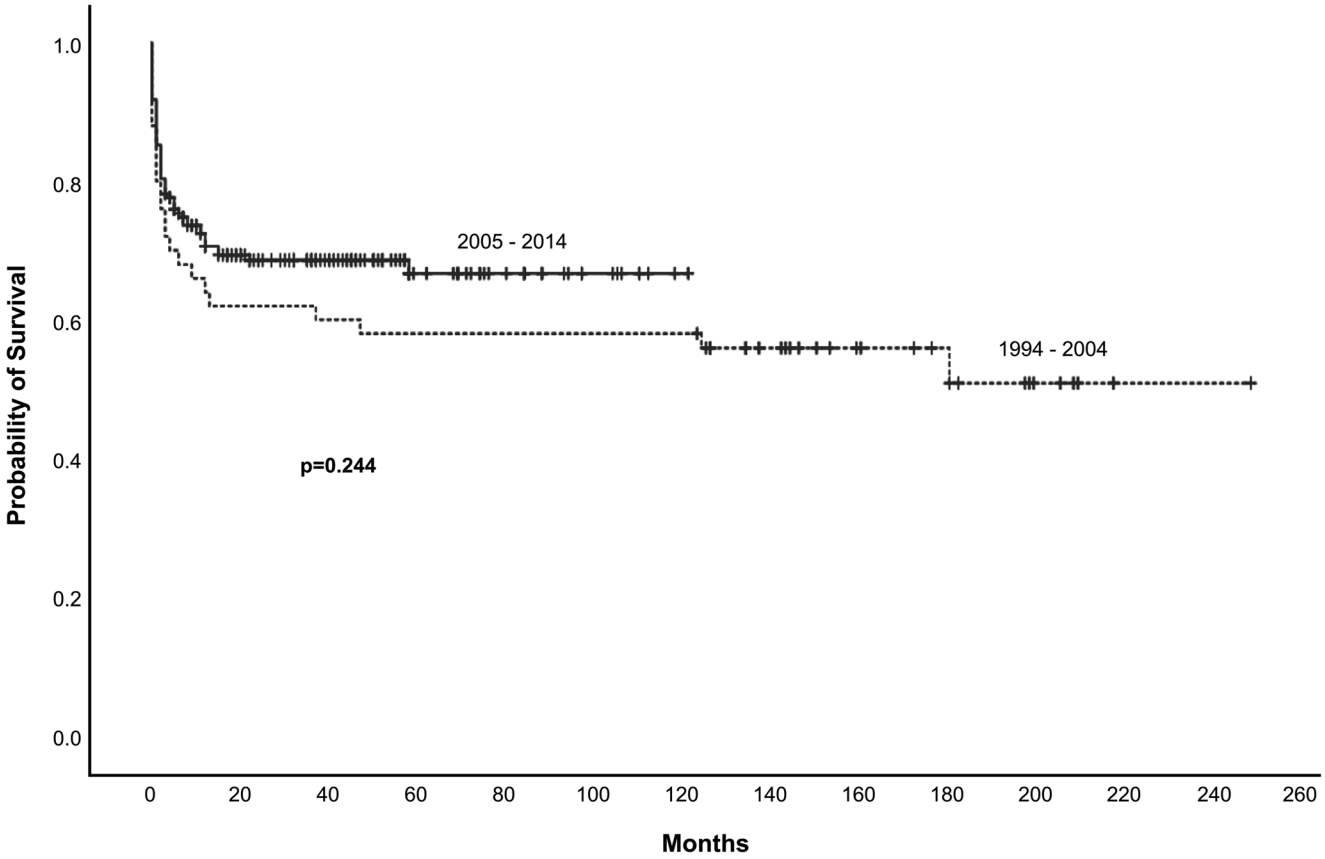


Fig. 4 The survival in SCID in relation to year of transplantation

institutions revealed that naive T cell (CD4+CD45RA+) counts as early as 6 months following the transplantation served as a prognostic factor for long-term immune reconstitution and finally survival [15].

Infection at the time of transplantation ($p = 0.006$), MMRD (haplo) and MUD donor transplants $p < 0.001$ are the most important factors significantly affecting outcome. Infection at transplantation, haplo, or MUD donors was found to be the major risk factors increasing mortality 2.2, 4.6 and 4.2 folds, respectively. So, these factors are the most significant factors contributing to poor outcome, decreasing survival probability. Late transplantation and implementation of myeloablative conditioning in infected patients at the time of transplantation could be the major factor behind this fact.

This is the first multicenter study in Turkey, with the largest data obtained from transplanted SCID patients. In conclusion, for better results, we need to have *early* diagnosis, earliest possible transplantation, and specialized teams. Newborn Screening (NBS) is of paramount importance in ensuring early diagnosis and timely transplantation. Fortunately, a prospective pilot NBS study has recently been started in Turkey, following a retrospective one.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflict of interest.

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