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ORIGINAL ARTICLE

Long-term immunological changes after corrective cardiac surgery

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Abstract

Infants with congenital heart disease (CHD) often undergo thymectomy during corrective cardiac surgery (CCS). The long-term immunological effects remain controversial, with concerns regarding increased susceptibility to infections, allergies, autoimmunity due to compromised immune tolerance mechanisms. This study aims to elucidate the long-term immunological effects of early thymectomy. We enrolled 22 patients who underwent thymectomy in infancy and were followed up in the Pediatric Allergy and Immunology Clinic at Marmara University. We performed demographic characteristics and detailed immunological evaluation, including immunoglobulins, vaccine responses, lymphocyte subset analyses, upregulation, proliferation of T cells and T-cell receptor excision circles (TRECs). Sixteen patients had a history of infection, including six serious infections, all in the first year. Lymphopenia was observed in 27% of patients, with a significant decrease in naive $CD4^+$ and recent thymic emigrant T cells counts and an increase in the proportion of memory T-cells, indicating premature immune senescence. Low levels of IgG, IgA and IgM were found in 36%, 40% and 22% of patients respectively. Vaccine responses were positive in 90% of patients. TREC levels were low in all 10 patients analysed. Seven of nine patients had normal proliferation.

Twenty-two percent of patients had allergic disease, and autoimmunity was not observed. Early thymectomy leads to permanent immunological changes that are indicative of early immunosenescence. It is recommended to preserve thymic tissue during surgery and requires long-term follow-up in terms of findings such as allergy and autoimmunity as well as infections due to impaired immune tolerance mechanisms.

KEYWORDS

congenital heart defect, early thymectomy, immunodeficiency, immunosenescence, lymphopenia, T cells, thymus

1 | INTRODUCTION

The thymus serves as the principal lymphoid organ and holds significant importance in the maturation of T cells. It creates an ideal microenvironment where lymphoid progenitors originating from the bone marrow undergo proliferation, T-cell receptor gene rearrangement and differentiation into fully functional T cells. These mature T cells initiate robust immune responses against pathogens or malignant cells. Furthermore, the thymus plays a critical role in negative selection, a process essential for eliminating T cells that potentially react against self-antigens, thus preventing autoimmune reactions.[1] Its peak activity is observed during prenatal development, infancy and early childhood, gradually declining over time.[2] Additionally, it contributes to the continuous replenishment of T cells, a process that persists at least until the sixth decade of life.[3] Immunosenescence is a complex process involving organ reorganization and multiple regulatory processes at the cellular level.[4] During this process, immune system function declines with age, and lack or delayed immune system maturation findings are not shared. Typically, some characteristic changes are observed during immunosenescence, including thymic involution, haematopoietic stem cell dysfunction, impaired naive/memory ratio of T and B cells, inflammation, accumulation of senescent cells, impaired novel antigen response, mitochondrial dysfunction, genomic instability and stress responses.[5–8]

In infants with congenital heart disease (CHD), sternotomy is often required and thymectomy is often performed to provide an open surgical field. The long-term effects of early thymectomy in conjunction with corrective cardiac surgery (CCS) have been a subject of ongoing debate. The early shift of immunosenescence, which is expected to occur with age, in patients with early thymectomy continues to be an important topic of debate. While many patients remain asymptomatic despite immunological findings indicating immunosenescence, the risk of future infection and autoimmune disorders may increase due to compromised central and peripheral tolerance mechanisms. In conclusion, removal of the thymus during CCS in early infancy leads to a significant reduction in the naive T-cell subset and the presence of markers indicative of premature immune senescence attributed to homeostatic proliferation and differentiation of naive T cells.[3,9] This phenomenon is believed to occur primarily in response to both self- and environmental antigens and it has been questioned whether early thymectomy increases the risk of autoimmunity and/or allergic disease. The limited number of studies present varying data on this topic.[10–15] The variability in findings among studies may partly stem from cohort heterogeneity concerning factors such as age at thymectomy, duration of follow-up post-thymectomy and residual thymic activity.[10–15] Importantly, it has been noted that thymic recovery can occur in some individuals.[16,17]

In recent years, immunologic outcomes of early thymectomy have become essential, with studies reporting long-term follow-up data.[11–13,15,17–24] Over time, there is a gradual development of CD4 and CD8 T-lymphocytopenia. This progression is marked by a decline in total T-lymphocyte levels and T-cell receptor excision circles (TRECs), alongside an increasing proportion of memory T-lymphocytes in the peripheral blood. Patients undergoing thymectomy show a higher frequency of naive T-lymphocytes expressing Ki67 than healthy individuals of the same age. This indicates enhanced peripheral replication in response to reduced thymic output.[15]

While early thymectomy does not result in infectious severe complications in the short term, its long-term consequences remain poorly understood. The absence of significant infections following early thymectomy might be attributed to the diverse pool of naive T-lymphocytes present at birth. These cells potentially offer protection against severe infections in the short and medium term. However, the impact of reduced thymic output throughout a lifetime on immune function and susceptibility to infections requires further investigation.[2]

Our study aims to elucidate the clinical and immunologic implications of thymectomy performed during CCS in early childhood. Moreover, this study represents the first comprehensive report from Turkey, providing detailed insights into the immunological status and long-term follow-up outcomes after early thymectomy.

2 | MATERIALS AND METHODS

2.1 | Data collection

2.1.1 | Subject characteristics

Twenty-two patients who underwent thymectomy during CCS in early infancy were enrolled in this study. Patients followed up at the Paediatric Allergy and Immunology Outpatient Clinic of Marmara University were included. Detailed descriptions of patients and laboratory data were collected from patient files. Records of total and partial thymectomy were obtained from the patients' operative notes. For patients without operative notes, information was obtained from the surgeons who performed the procedures. The study protocol was approved by the local ethics committee of Marmara University, and written informed consent was obtained from all parents and, where possible, from the patients themselves. Patients with Down or DiGeorge syndrome and other immune deficiencies related to thymic development defects were excluded from the study.

2.1.2 | Antibodies and flow cytometry

Peripheral blood lymphocyte subset analyses, upregulation and proliferation assays were performed by flow cytometry and compared with age-matched controls.[25–29] To determine detailed lymphocyte subsets, the following monoclonal antibodies (mAbs) were used: Fluorescein isothiocyanate (FITC)-conjugated CD3 (UCHT1, BC, FRA), Allophycocyanin (APC)-Alexa Fluor 700 (APC-A700) CD4 (13B8.2, BC), Krome Orange (KO) CD45 (J33, BC), Alexa Fluor 750 (APC-A750) CD45RA (2H4DH11LDB9, BC), Phycoerythrin (PE) CD197 (CCR7) (G043H7, BC), Pycoerythrin-Cyanin 7 (PC7) CD8 (SFCI21Thy2D3, BC), APC-A700 CD14 (RMO52, BC), PE CD16 (3G8, BC), Pycoerythrin-Cyanin 5.5 (PC5.5) CD56 (N901, BC), APC-A750 CD19 (J3-119, BC), Pacific Blue (PB) CD20 (B9E9, BC), PB CD21 (BL13, BC), PB CD31 (5.6E, BC), PC5.5 CD38 (LS198-4-3 BC), Phycoerythrin-Texas Red-x (ECD) CD45RO (UCHL1, BC), FITC IgD (IA6-2, BC), PB CD4 (RPA-T4, Biolegend), FITC CD45RA (HI100, Biolegend), PC5.5 CD25 (B1.49.9, BC), APC-A750 CD127 (R34.34, BC) PE CD183 (CXCR3) (G025H7, Biolegend), APC CD185 (CXCR5) (J252D4, BC), PC7 CD196 (CCR6) (B-R35, BC), PE CD279 (PD-1) (PD1.3, BC). For lymphocyte subset analysis, 100 μL of whole blood was incubated with mAbs against surface markers for 20 minutes in the dark at room temperature. Red cells were lysed and washed before acquisition. Cells were acquired by Navios EX cytometer (Beckman Coulter) and analysed by FlowJo software (TreeStar, Ashland, Ore). T lymphocyte upregulation and proliferation assay were performed by stimulation with phytohemagglutinin (PHA) and anti-CD3/CD28 (1 μg/mL each) 96-well plates for 3 days, following isolated PBMCs labelling with CellTrace Violet (Thermo Fisher). After the stimulation, cells were stained with APC-A700 CD4 (13B8.2, BC), PC7 CD8 (SFCI21Thy2D3, BC) and PC5.5 CD25 (B1.49.9, BC).[30,31] Stained cells were acquired by Navios EX cytometer (Beckman Coulter) and analysed by FlowJo software (TreeStar, Ashland, Ore).

2.2 | Study of TREC/KREC copy numbers

Samples (10 mL) of peripheral blood were obtained from patients. DNA was isolated using DNA Extraction and Purification Kits (QIAGEN) as described by the manufacturer. For the study, each sample was aliquoted at 10 ng/μL. Absolute copy numbers were determined using plasmids carrying the house-keeping gene T cell receptor alpha constant (TRAC) and the TREC/KREC encompassing gene regions housekeeping. The plasmid copy number was calculated according to the plasmid size, and final copy numbers were obtained by 10-fold dilutions (1×10^6–1×10^1). TREC/KREC copy numbers were determined by real-time qPCR in a LightCycler 480 system (Roche). The primers and probes used to amplify the regions of interest have been previously described.[32] The PCR conditions were as follows: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 s. The samples had a crossing cycle. Each sample was evaluated in duplicate.[33,34] The number of TRECs or KRECs per 10^6 peripheral blood mononuclear cells was calculated using the following formula:

$$\text{TREC and KREC copy numbers} = \frac{\text{Mean of TREC or KREC quantity}}{\text{Mean of TRAC quantity} / 2} \times 10^6$$

(As there are two *TRAC* gene copies in each cell, the mean *TRAC* quantity was divided by 2).

2.3 | Statistics

Statistical analysis was conducted using the Jamovi 2.3.26 version (The Jamovi Project, Australia). The data were expressed as mean and standard deviation

if distributed normally, otherwise with median and interquartile range (IQR) of 25%–75%. Continuous variables between groups were compared using the Student *t*-test when they followed a normal distribution, and the Mann–Whitney *U* test was used for variables that did not follow a normal distribution. The categorical variables between groups were compared using the chi-square test. A *p*-value below 0.05 was considered statistically significant within a 95% confidence interval. Graphs are produced by GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA) and Adobe Illustrator 25.2.1 (Adobe Inc., USA).

3 | RESULTS

3.1 | Patient characteristics

Twenty-two patients were evaluated, including 16 males (72%) and 6 females (28%). The median current age of the patients was 84.5 months (IQR 40.3–156). The median follow-up age was 20 months (IQR 13.3–34). The age at cardiac surgery was less than 12 months in all, except one patient (P5). Twelve patients (54%) had upper respiratory tract infection (URTI), 11 had (50%) lower respiratory tract infection (LRTI) and 6 (27%) had both URTI and LRTI. The median age of infection symptom was 0 months (IQR 0–2.7). Six patients (30%) had a history of severe infections (P1: hepatic abscess, P3: pneumonia, P4: sepsis, P7: diarrhea, P10: pneumonia, P17: pneumonia). All severe infections occurred in the first year. EBV, CMV infections, candidiasis and other opportunistic infections were not observed. Five patients (22%) had allergic disease (P6: asthma, P12: allergic rhinitis, drug allergy, P13: asthma, P19 and P21: asthma, allergic rhinitis) and none had autoimmunity. Aeroallergen sensitization was present in all patients with allergic disease. All patients were alive at the time of the study (Table 1). Total thymectomy was performed in 18 patients, and partial thymectomy in 4 patients. The characteristics of the patient's cardiac defects and thymectomy are shown in Table 2.

TABLE 1 Characteristics of the patients.

Characteristics	
Gender F/M (*n*, %)	6 (27)/16 (73)
Current age (month) (IQR, %25–75)	84.5 (116; 40.3–156)
Age of symptom onset (month) (IQR, 25%–75%)	0 (2.25; 0–2.25)
Follow-up time (month) (IQR, 25%–75%)	20 (20.8; 13.3–34)
Date of cardiac operation (month) (IQR, 25%–75%)	0 (3.5; 0–3.5)
Total (*n*, %)/partial (*n*, %) tymectomy	18 (82)/4 (18)
Upper RTI (*n*, %)	12 (60)
Lower RTI (*n*, %)	11 (50)
Infection in the first year of life (*n*, %)	14 (63)
Infection after the first year of life (*n*, %)	9 (40)
Severe infection (*n*, %)	7 (31)
Allergic disease (*n*, %)	5 (22)
Autoimmunity (*n*, %)	0

Abbreviations: IQR, interquartile range; RTI, respiratory tract infection.

3.1.1 | Immunological investigations

Immunological evaluation was performed at a median of 79 months (IQR 20.3–137) after thymectomy. Nine patients (27%) had lymphopenia. Eight patients (36%) had low IgG levels (median 774 [IQR 481–1035]), nine patients

TABLE 2 Cardiac defects and thymectomy type of patients.

Patients	Type of cardiac defects	Type of TX	Age at TX, months
1	TGA, VSD, AoH	Total	1
2	VSD, CoA	Total	3.5
3	BAT	Total	3.5
4	CoA, VSD, ASD	Total	1
5	SV	Partial	41
6	BAT	Partial	2
7	SV	Total	0
8	RAI, DORV, HLHS	Total	4
9	LVH, PS, AS	Total	0
10	TOF	Total	0
11	CoA, VSD, ASD	Total	0
12	BAT	Total	0
13	CoA	Total	0
14	BAT, ASD	Total	0
15	BAT	Total	0
16	HLHS	Total	5
17	TOF	Partial	0
18	CoA	Total	0
19	ASD, VSD	Total	8
20	TA, BTrV	Partial	0
21	TGA, VSD	Total	1
22	CoA	Total	3

Abbreviations: AoH, aortic hypoplasia; AS, atrial stenosis; BTrV, bicuspid truncal valve; CoA, aortic coarctation; DORV, double outlet right ventricle; HLHS, hypoplastic left heart syndrome; HRHS, hypoplastic right heart syndrome; LVH, left ventricular hypertrophy; PA, pulmonary atresia; PS, pulmonary stenosis; RAI, right atrial isomerism; SV, single ventricle; TA, truncus arteriosus; TOF, tetralogy of fallot; TvA, tricuspid valve atresia; TX, thymectomy.

(40%) had low IgA levels (median 51 [IQR 30.5–122]), and five patients (22%) had low IgM levels (median 94.5 [IQR 56.5–110]) (Figure 1A). Positive responses to protein vaccines were detected in 20 patients. Of the 18 patients for whom isohemagglutinin titers could be assessed, 14 had protective titers (Table S1).

Flow cytometry analysis revealed low $CD3^+$ T-cell counts in 14 patients (63%), low $CD4^+$ T-cell in 14 patients (63%), low $CD8^+$ T-cell counts in 11 patients (50%), high $CD8^+$ T-cell count in one patient. The $CD4^+/CD8^+$ T-cell ratio was inverted in 9 patients (41%). Percentage of naive $CD4^+$ T-cells ($CD4^+$ $CD45RA^+$) was low in 13 patients (59%), whereas percentage of memory $CD4^+$ T-cells ($CD4^+CD45RO^+$) was high in 16 patients (72%) (Table S1, Figure 1B).

Accordingly, percentage of effector memory $CD4^+$ T cells (EMCD4, $CD4^+$ $CD45RA^-$ CCR7−) increased in 19 patients. All patients had normal percentage of naive $CD8^+$ T cells ($CD8^+$ $CD45RA^+$) except for two patients (P1 and P13), whereas memory percentage of $CD8^+$ T cells ($CD8^+$ $CD45RO^+$) was high in three patients. However, percentage of effector memory $CD8^+$ T cells (EMCD8, $CD8^+$ $CCR7^-$ $CD45RA^-$) was high in 10 patients (45%) (Table S1). The percentage of recent thymic emigrant T cells (RTE, $CD4^+$ $CD45RA^+$ $CD31^+$) decreased in all except three patients (P4, P8 and P20) (Table S1, Figure 1B).

TREC levels were analysed in 10 patients and found low in all (median 360 [IQR 249–667]) (Table S1).

Five patients (23%) showed increased levels of $CD19^+$ B cells. The percentages of unclass-switched memory B (UCSMB) cells and class-switched memory B (CSMB) cells were low in three (14%) and six patients (27%) respectively. $CD16^+$ 56^+ NK-cell counts were increased in six patients (27%). The detailed results are presented in Table S1 and Figure 1B.

We evaluated the T-cell proliferation in nine patients (41%). Normal T-cell proliferation and upregulation of CD25 with anti-CD3/CD28 and PHA were detected in $CD4^+$ T cells and $CD8^+$ T cells of patients except two (P1, P10) compared to age-matched healthy controls (Table S1 and Figure 1C). P10 shows decreased T-cell proliferation and decreased upregulation of CD25 with PHA in $CD8^+$ T cells and $CD4^+$ T cells in Figure 1D.

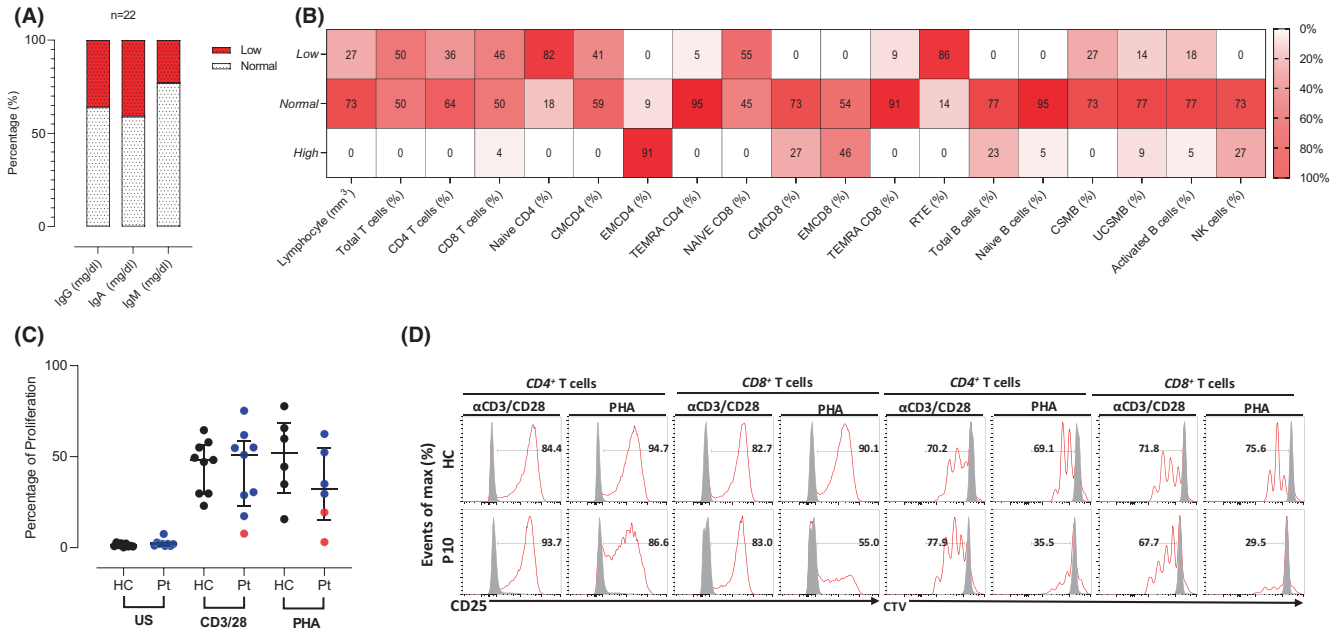

FIGURE 1 Immunological features of the patients. (A) Serum Ig G, M and A levels of patients. The proportion of levels is presented as decreased or normal (B) The heat map illustrates the lymphocyte subsets of the patients. According to the healthy age-matched reference values for the indicated parameters, the proportion of cells is shown as high, low or normal (CM $CD4^+$ T cells; Central Memory $CD4^+$ T cells; EM $CD4^+$ T cells, Effector Memory $CD4^+$ T cells; TEMRA $CD4^+$ T cells, exhausted $CD4^+$ T cells; CM $CD8^+$ T cells, Central Memory $CD8^+$ T cells; EM $CD8^+$ T cells, Effector Memory $CD8^+$ T cells; TEMRA $CD8^+$ T cells, exhausted $CD8^+$ T cells; RTE cells, Recent Thymic Emigrant cells; CSMB cells, Class Switched Memory B cells; UCSMB cells, Unclass Switched Memory B cells; NK cells, Natural Killer cells. (C) Comparison of T-cell proliferation assays in patients and healthy controls. T-cell proliferation assay with anti-CD3/CD28 (P1, P2, P3, P4, P7, P10, P13, P14 and P20) and PHA (P1, P4, P7, P10, P13 and P20) stimulation of patients compared to healthy controls, no significant difference (Mann–Whitney *U* test). Healthy controls are shown in black, patients with normal proliferation in blue, and patients with low proliferation in red dots. The experiment was repeated twice at different time points. (D) Representative flow cytometric analysis of percentages of CD25 and proliferation in $CD4^+$ and $CD8^+$ T cells of P10 and age-matched healthy control with unstimulated and stimulated conditions (anti-CD3/CD28 and PHA). Pt, patients; HC, healthy controls; PHA, phytohemagglutinin; αCD3/CD28, anti-CD3/CD28.

When the immunological profiles of patients under and over 5 years old were evaluated, we found that patients over five had significantly lower levels of naive CD4, central memory (CM) CD4 and RTE and higher levels of EMCD8 ($p=0.01$, $p=0.006$, $p=0.036$, $p=0.03$, respectively) (Figure 2A–D). Patients with low naive CD4, CMCD4 and RTE had a significantly longer duration after cardiac surgery ($p=0.01$, $p=0.001$, $p=0.02$, respectively) (Table 3).

When immunological profiles of patients with partial and total thymectomy were compared, naive CD4 was significantly lower, and memory CD4 was significantly higher in patients with total thymectomy ($p=0.01$, $p=0.04$, respectively) (Figure 3A, B).

3.1.2 | Genetics evaluations

We evaluated 17 patients with fluorescence in situ hybridization analysis to rule out chromosome 22q11.2 deletion syndrome; no deletion was detected in all patients. Nine patients underwent next-generation sequencing for syndromic combined immunodeficiency, revealing no pathogenic variant.

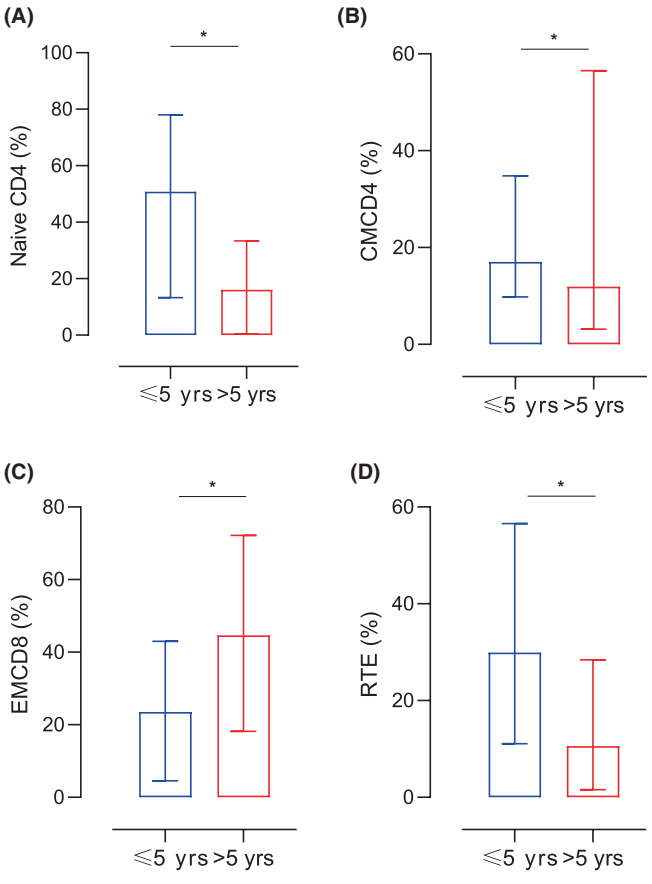

FIGURE 2 Comparison of Naive CD4, CMCD4, EMCD8 and RTE cell percentages in patients under and over 5 years of age. Analysis of (A) percentage of Naive CD4, (B) percentage of CMCD4, (C) percentage of EMCD8, (D) percentage of RTE. *$p < 0.05$, Fisher exact test.

4 | DISCUSSION

The thymus ensures its replenishment with naive cells with a vast and diversified repertoire of receptors but limited self-reactivity.[9] In addition to the loss of a lymphoid organ necessary for T-cell education, early thymectomy causes a transient reduction in T-cell counts. It may increase rates of homeostatic T-cell proliferation.[17,21,22] This study details the effects of early thymectomy on the immune system's long-term functioning, and it reveals lower levels of total T cell, $CD4^+$, $CD8^+$, RTE and TREC in children who had thymectomy during their early years of life.

A lack or limited population of oligoclonal pure T-lymphocytes at birth predisposes children to a major impairment of acquired immunity in congenital thymic aplasia, such as complete DiGeorge syndrome, which is indicated by a severe, persistent and opportunistic infection in infancy. Sixty-four percent of a sizable cohort of patients with partial DiGeorge syndrome experienced recurrent or severe infections, and 7% developed an autoimmune disease beginning at age 7.8 years.[1] The absence of significant infections following neonatal thymectomy may be explained by the diverse pool of naive T-lymphocytes present at birth following normal foetal thymic development and function, which is present before thymic ablation and age-related decline. On the other hand, management recommendations for DiGeorge syndrome include immunological monitoring, antimicrobial prophylaxis, vaccination, and immunoglobulin replacement when necessary. In contrast, no consensus exists regarding treating acquired thymic aplasia.[2]

The evaluation of early thymectomy's long-term implications is only getting started. Previous research indicates that despite immunological indications indicative of ageing immunity, a large number of individuals continue to show no symptoms.[2] In two studies, hospitalization due to the cause of infection and mean antibiotic duration were significantly higher.[35,36] In our study, 16 patients (72%) had a recurring history of infection, and within the first year, 15 out of the 16 patients (93%) experienced symptoms. Furthermore, 6 out of 8 patients (75%) with a history of serious infection (requires hospitalization) had an infection within the first year. Although the incidence of infection appears to be high according to previous studies, these infections occurred mostly within the first year. The infection rates may be explained by frequent postoperative hospitalization and general health problems related to surgery, like feeding problems.

TABLE 3 Naive CD4, CM CD4 and RTE cells are lower in patients with longer time since CCS.

	Time after the CCS (months), Median (min–max)	*p* value
Naive CD4 (low) Naive CD4 (normal)	97.5 (5–174) 5.5 (5–36)	0.01
CMCD4 (low) CMCD4 (normal)	145 (74–174) 27 (5–118)	0.001
RTE (low) RTE (normal)	90 (5–174) 27 (5–118)	0.02

Note: $p < 0.05$ is statistically significant (Mann–Whitney *U* test).

Abbreviations: CCS, corrective cardiac surgery; CMCD4, central memory $CD4^+$ T cell; RTE, recent thymic emigrant T cells.

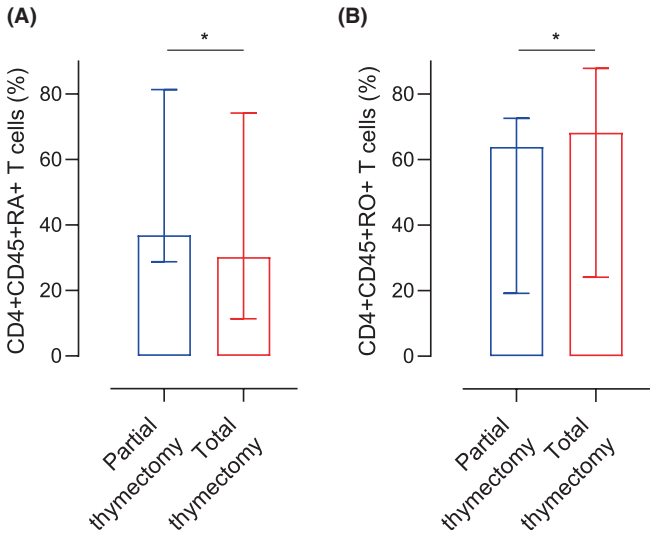

FIGURE 3 Comparison of the percentages of Naive CD4 and memory T cells in patients with partial and total thymectomy. Analysis of (A) percentage of $CD4^+$ $CD45RA^+$ T cells, (B) percentage of $CD4^+$ $CD45RO^+$ T cells. *$p < 0.05$, Fisher exact test.

It is now known that naive T-lymphocyte and TREC levels gradually decrease after thymectomy, whereas peripheral memory T-lymphocyte fraction rises and resembles the previously reported immunosenescent phenotype.[11,13,19] However, if thymic regeneration occurs, these alterations are reversible.[16,17,21] Similarly, our patients' most decreasing cell groups were naive CD4 and RTE (82% and 86%, respectively). Generally, TREC levels were lower in thymectomized patients than in controls.[11,13,15,17,36–40] In our study, TREC levels were low in all patients who could be measured. As thymic exports stop or decrease, the peripheral T-cell pool multiplies, renews the peripheric area, and gradually gains the effector/memory phenotype as seen in lymphopenic conditions and ageing.[41–44] As a result, naive T cells gradually disappear, and the effector/memory phenotype predominates. Our study's most increasing T-cell subset was EMCD4 cells (86%). Naive T-cell function is impaired after neonatal thymectomy, but it is unclear whether it is restored by thymic tissue regeneration in later stages of life.

Although functional thymic tissue regeneration after neonatal thymectomy and restoration of the naive $CD4^+$ T-cell compartment occurs in most children in advanced stages of life, a subgroup of these children shows no signs of restoring thymic output.[16] Thus, the literature has a range of findings about the longevity of immune changes. Nonetheless, most studies indicate that following thymectomy, cellular-mediated immunity gradually and persistently declines.[11,13,15,24,37,38] The most extended follow-up study to date of 18 years demonstrated a decrease in quantitative naive T-lymphocytes, contraction and skewing of TCR repertoire reflecting a decrease in the peripheral pool of naive T-lymphocytes with an increase in pre-existing T-lymphocyte clones and a possible decrease in T-lymphocyte proliferative ability indicated by shorter telomere length.[11] To demonstrate the changing immunological profile over time, we compared immunological findings in patients under 5 years of age and older. Patients over the age of 5 had lower levels of naive T cells, CMCD4, RTE and higher levels of EMCD8 cells compared to patients under age 5, suggesting that the results of early thymectomy could be permanent. However, no statistically significant difference was found between the frequency of infection in the two groups.

In the limited studies that have assessed immunoglobulin abnormalities after thymectomy, decreased levels of IgG, IgA and IgG1 subclasses have been observed,[12,24,45] and variable results in specific antibody responses[35,45–47] have been reported. However, changes in B-lymphocyte subsets have not been adequately investigated. In our study, IgG and IgA levels were low in approximately 40% of the patients, according to the literature. When B-cell subgroups were analysed, CSMB and UCSMB cells were low in some patients (27% and 14%, respectively). However, protein vaccine responses and isohemagglutinin titers were largely positive, supporting the absence of functional impairment of the humoral response in patients.

Most studies demonstrated whole or more than 90% thymus removal; however, several included total and partial thymectomy in the same group and still noted a sustained decrease in naive T cells, $CD4^+$, $CD8^+$ and total T-cell counts. In our patients, the total thymectomy rate was high, and in the few patients who underwent partial thymectomy, naive T cells were significantly higher, and memory T cells were significantly lower.

Following thymectomy, there is an observed increase in the proportion of Treg cells within the $CD4^+$ T-lymphocyte pool. This increase may be attributed to the preferential proliferation of Treg cells. Additionally, there is a decrease in naive Treg cells characterized by $CD4^+$ $CD25^+$ CD127low $CD45RA^+$ phenotype, along with an increase in memory Treg cells characterized by $CD4^+$ $CD25^+$ CD127low $CD45RO^+$ phenotype. These memory Treg cells exhibit greater suppressive potential, suggesting a compensatory mechanism for peripheral tolerance in response to the lack of central tolerance following thymectomy.[11] The incidence of autoantibodies has been identified in patients undergoing early thymectomy, and the detection of antinuclear antibodies or antineutrophilic cytoplasmic antibodies has increased with age in half of patients undergoing a cohort thymectomy.[24] However, this finding has not been repeated in all studies. The accumulation of memory T lymphocytes and the switch from TH1 to TH2 cytokine profiles explain the promotion of humoral immunity, autoantibody production and the potential to develop autoimmunity. In our study, three patients had autoantibody-positive, but we did not identify a clinical autoimmunity. Clinical autoimmune disease is not common due to the preferential proliferation of Treg cells and an increase in post-thymectomy rates that suppress autoreactivity.[24] However, since autoantibodies may appear long before clinical symptoms develop, further monitoring is needed to determine whether the clinical autoimmune disease develops in the advanced stages of life.

We advocate that all patients undergoing CCS early in life should be screened for acquired thymic aplasia. In light of the literature and based on our study results, surgical procedures should aim to preserve at least some thymic tissue. Remarkably, immunological findings persist after early childhood in these patients, and it is crucial to monitor clinical (infection, allergy, autoimmunity) and immunological findings in the long term.

AUTHOR CONTRIBUTIONS

S.B. and S.B.E. conceptualized and supervised the study. M.C.C., A.B., G.O., Y.Y.N. and O.H.N. performed the experiments. S.B.E., R.A., R.B., M.Y.A., T.K., S.C., E.Y.G., S.B., N.O., E.K.A., A.O. and S.B. provided patient care, collected samples and clinical data. S.B.E. and S.B. wrote the paper. All authors reviewed and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The data generated during the study are included in this published article.

ETHICS STATEMENT

The study was approved by the Ethics Committee of Marmara University, School of Medicine (09.2022.32).

CONSENT TO PARTICIPATE

Informed consent was obtained from all individuals.

CONSENT FOR PUBLICATION

Informed publication consent was obtained from all participants.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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