TREC and KREC values in patients with congenital heart defects — neonatal screening data

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Abstract

Relevance. Children with congenital heart disease (CHD) are at high risk of infectious complications with unfavorable outcomes, which is associated with inadequate immune responses. TREC and KREC are recognized biomarkers of T- and B-cell lymphopoiesis.

Objective of the study: to evaluate the number of circular DNA segments — TREC and KREC in children with congenital heart defects.

Materials and methods. The study used data from neonatal screening for primary immunodeficiencies in the Stavropol region from January 1, 2023, to June 30, 2024. An analysis of TREC and KREC was conducted in 43 newborns with CHD compared to healthy infants.

Results. The levels of TREC and KREC in children with CHD were lower than in healthy children. A correlation was established between TREC levels and absolute lymphopenia, as well as the development of infectious complications. A decrease in KREC was identified in infants with CHD and genetic syndromes. It was shown that a TREC level of less than 650 copies per 10^5 cells may be a predictor of the development of infectious complications in newborns with CHD.

Conclusions. The reduction in the number of TREC and KREC copies in neonatal screening allows for the identification of children with CHD at high risk of infection, opening potential opportunities for preventive therapy.

Keywords: congenital heart defects, TREC, KREC

Conflict of interests:
The authors declare no conflict of interest.

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Показатели TREC и KREC у пациентов с врожденными пороками сердца. Данные неонатального скрининга

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Аннотация

Актуальность. Дети с врожденными пороками сердца (ВПС) имеют высокий риск инфекционных осложнений с неблагоприятным исходом, что связано с неадекватным иммунным ответом. TREC и KREC являются признанными биомаркерами Т- и В-клеточного лимфопоэза у новорожденных.

Цель исследования: оценить количество кольцевых участков ДНК — TREC и KREC у детей с врожденными пороками сердца.

Материалы и методы. При проведении исследования использовались данные неонатального скрининга на первичные иммунодефициты в Ставропольском крае за период с 01.01.2023 по 30.06.2024. Осуществлен анализ TREC и KREC у 43 новорожденных с ВПС по сравнению со здоровыми младенцами.

Результаты исследования. Показатели TREC и KREC у детей с ВПС были ниже, чем у здоровых детей. Установлена связь уровней TREC с абсолютной лимфопенией, развитием инфекционных осложнений. Определено снижение KREC у младенцев с ВПС и генетическими синдромами. Установлено, что уровень TREC менее 650 копий на 10^5 клеток может быть предиктором развития инфекционных осложнений у новорожденных с ВПС.

Выводы: уменьшение числа копий TREC и KREC в неонатальном скрининге позволяет выявить детей с ВПС с высоким риском инфицирования, открывая потенциальные возможности для профилактической терапии.

Ключевые слова: врожденные пороки сердца, TREC, KREC

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Relevance. Excision rings of T-cell receptor rearrangement genes (TREC) and k-deletion excision circles (KREC) are formed during differentiation of immune cells in the territory of central organs of immunopoiesis at the stage of forming their antigen-recognizing repertoire and are markers of newly formed T- and B-lymphocytes [1, 2]. Multiplex real-time PCR assay of TREC and KREC is used in neonatal screening in many countries of the world to diagnose severe combined immune deficiency (SCID) and other congenital immune errors in newborns [1, 2]. In addition to TCIN, TREC detection can identify other forms of T-cell lymphopenia (TCL), including Di Giorgi, CHARGE, Jacobsen, Louis-Bar, Wiskott-Aldrich McKusick, Noonan, Down syndromes, as well as secondary TCL in infants with cardiac, gastroin-

testinal, neonatal leukemia, and profound prematurity syndromes [3, 4, 5, 6]. It has been shown that non-TREC TCL are 3-4 times more common than TREC [3, 7]. A high percentage of children with abnormal TREC indices have congenital heart disease (CHD) [3, 7].

It is known that infants with CHD suffer from frequent infections with a high risk of complications and fatal outcomes [8]. Clinical trials demonstrate low levels of T-lymphocytes and their subpopulations in children with CHD, which confirms the important role of TCL in the development of infectious complications [8, 9]. It has been previously shown that reduced TREC copy number in newborns may be associated with heart defects [3, 5, 7, 10]. However, the prevalence of T-cell lymphopenia in children with CHD has not been fully determined.

STUDY OBJECTIVE: evaluate the results of neonatal screening (TREC, KREC) in children with congenital heart disease.

MATERIALS AND METHODS. We conducted a single-center retrospective cohort study including infants with diagnosed structural CHD born in Stavropol Krai between 01.01.2023 and 30.06.2024. Patients were selected on the basis of Stavropol Clinical Perinatal Centers #1 and #2, as well as the Department of Pathology of Newborns and Prematurity of the Regional Children's Clinical Hospital of Stavropol city. The diagnosis of congenital heart disease was determined in accordance with clinical guidelines on the basis of clinical and instrumental data taking into account ICD-9. The archival case histories of patients were analyzed. Infants with CHD born at gestational age of more than 34 weeks were included in the study; infants with other causes of secondary lymphopenia, including profound prematurity, GI malformations, neonatal leukemia, and those born to HIV-infected mothers and mothers who received immunosuppressive therapy during pregnancy were excluded.

The results of TREC and KREC in 43 children with CHD and 100 healthy children were obtained

during neonatal screening and provided by the neonatal screening laboratory of the Stavropol Krai Clinical Perinatal Center (SKCCPC). Blood was collected at the age of 24-48 h from preterm neonates and 144-168 h from premature neonates (Order of the Ministry of Health of the Russian Federation from 21.04.2022 № 274N.) The study was approved by the Local Ethical Committee of StSMU. Parents of patients signed informed consent for participation in the study.

TREC and KREC were determined at the research center of the S. V. Ochapovsky Krai Clinical Hospital No. 1 (Krasnodar) by real-time quantitative polymerase chain reaction for analysis of dried blood spots. TK-SMA" test systems of Generium were used.

A threshold level of TREC and KREC of less than 100 per 100,000 cell copies was considered criteria for a positive result for SCID and other inborn errors of the immune system according to the screening procedure [11].

If low levels of TREC/KREC were obtained, the first stage of confirmatory diagnostics was performed on a test form in the Federal State Budgetary Scientific Institution "Medical and Genetic Research Center named after Acad. N. P. Bochkov". In newborns with abnormal TREC/KREC, according to the retest data, extended immunophenotyping (IPT) was performed in the immunologic laboratory of the Dm. Rogachev MDRC and molecular genetic study with determination of 22q11.2 and 10p14 deletion, as well as exome (NGS) and, if necessary, full exome (WGS) sequencing in the N. P. Bochkov Medical and Genetic Research Center. Patients with one or more IFT indices below the reference values (CD3 < 1500 kL/μL, CD3CD45RA < 60%, CD19 < 400 kL/μL) in the absence of pathogenic variants in the primary IDS genes were referred to the secondary lymphopenia group [11].

Statistical analysis of the data was performed using Statistica 10 software. Demographic data as well

as excision ring indices of rearrangement genes were presented using descriptive statistics in the form of median and interquartile range. The Mann-Whitney U-criterion was used to compare TREC and KREC values. ROC curve analysis was used to assess the significance of TREC/KREC as predictors of infectious complications. Values of $p < 0.05$ were considered statistically significant.

STUDY RESULTS. 43 infants (25 boys and 18 girls) with heart defects enrolled in a neonatal screening program for 1.5 years were included in the study. Cyanotic heart defects were verified in 5 (11.6%) infants, including tetrad of Fallot (2), single ventricle of the heart (1), and transposition of the main vessels (2). Among noncyanotic defects, aortic coarctation was diagnosed in 7.9 %, aortic valve stenosis in 10.5 %, atrial septal defects in 21.1 % and interventricular septal defects in 44.7 %, atrioventricular communication (7.9 %), and ductus arteriosus (7.9 %). In 9 (20.9 %) cases CHDs were found in the structure of genetically determined diseases: Edwards syndromes — 4.6 %, Aper syndromes — 2.3 %, Down syndromes — 13.9 %. Surgical treatment was performed in 15 (34.9%) patients.

40 children were born prematurely (gestational age ⩾37 weeks) and three were moderately premature (32-36 weeks). 9 (20.9%) children had low birth weight relative to gestational age. Hypoxic-ischemic encephalopathy (HIE) of moderate severity was diagnosed in 28 (65.1%) newborns, and severe HIE with development of IVH was diagnosed in 2 (4.7%). Serious infections developed in 28 (65.1%) infants, including pneumonia in 25 (58.1%), bronchiolitis/bronchitis in 1 (2.3%), necrotizing enterocolitis in 1 (2.3%), urinary tract infections in 5 (11.6%), and skin infections in 2.3%. (1).

Abnormal TRECs (less than 100 copies per 10^5 cells) were obtained in 2 children, representing 22.2% of 9 cases of secondary lymphopenia verified in SK within 1.5 years (01.01.23 to 01.07.24).

Patient 1. He was born at 39 weeks of gestation, with signs of asphyxia, grade 2 DN, hospitalized in the ORIT. A CHD was diagnosed in the newborn period: pulmonary artery stenosis, a functioning oval window. In the framework of neonatal screening low KREC number — 70 copies per 100 thousand leukocytes was detected and confirmed by retest. The hemogram at the age of 3 weeks of life revealed moderate lymphopenia — 2900 cells/μL. According to immunophenotyping data, normal content of CD3 — 2700 kl/μl, CD4 — 2300 kl/μl, CD8 — 490 kl/μl, significant decrease of CD19 — 50 kl/μl, and signs of transient infantile hypoimmunoglobulinemia (IgA — 0 g/L, IgG — 3.16 g/L, IgM — 0.51 g/L) were determined. Molecular genetic study revealed no clinically significant mutations by full-exome sequencing. The child underwent neonatal pneumonia, and there were no serious infectious diseases in the subsequent period. Lymphopenia resolved at the age of 3 months.

Patient 2. The boy was born prematurely at the 34th week of gestation with a weight of 1490 g and an Apgar score of 4. At the 22nd week of pregnancy, cordocentesis and karyotyping were performed, and Edwards syndrome was verified. At birth the condition was severe due to cardiovascular insufficiency, DN 2-3rd stage, hypoxic-ischemic encephalopathy 2nd stage. CHD of high complexity category was confirmed: common atrioventricular canal. The patient had a ASD (3.5 mm), VSD (5 mm), PDA (2.5 mm) with signs of pulmonary hypertension. The results of NS after the post-conceptional age of 37 weeks revealed low KREC — 96 copies per 100,000 cells. IFT results revealed normal CD3 counts of 3474 cells/μL, CD3CD4CD45RA counts of 1844 cells/μL, and low CD19 counts of 132 cells/μL. No clinically significant mutations were detected according to WGS data.

During 4 months of observation there were continuously recurrent bacterial infections (pneumonia, omphalitis, enterocolitis, otitis media, pyoderma), lack of weight gain, cytopenic syndrome (platelet count 50-90*10^9/l, anemia of 2-3rd degree), need for continuous antibacterial and antifungal therapy. During the monitoring of humoral immunity factors the decrease of serum immunoglobulins was determined (IgA — 0.1 g/l, IgG — 2.9 g/l, IgM — 0.35 g/l). He is in the intensive care unit of SKCPC, prognosis is doubtful.

When evaluating the screening results in the general CHD group, a statistically significant decrease in TREC — 1198 [698; 1717], ($p<0.05$) and KREC — 954 [587; 1253] copies per 10^5 cells, $p<0.01$ compared to healthy children was revealed (Fig. 1, 2). A decrease in TREC values in newborns with cyanotic CHD, genetic diseases in combination with CHD was determined, as well as in children with lethal out-

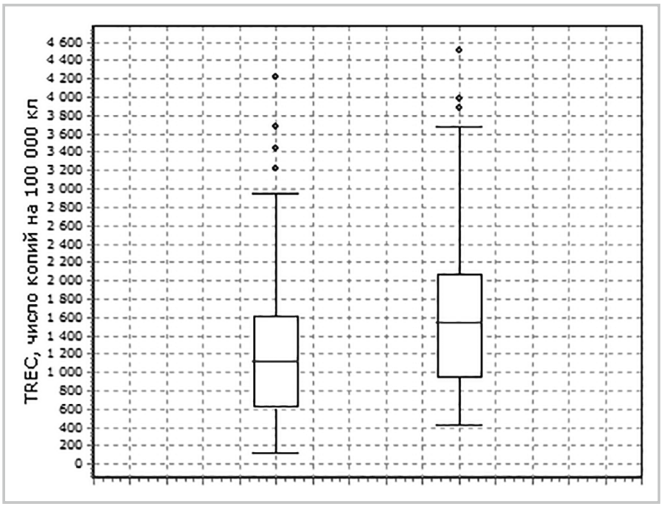

Fig. 1. **TREC indicators in children with congenital heart defects (illustration by the author)**
Рис. 1. **Показатели TREC у детей с врожденными пороками сердца (иллюстрация автора)**

Note: p — differences compared to healthy children (Mann — Whitney test)

Примечание: p — различия по сравнению со здоровыми детьми (критерий Манна — Уитни)

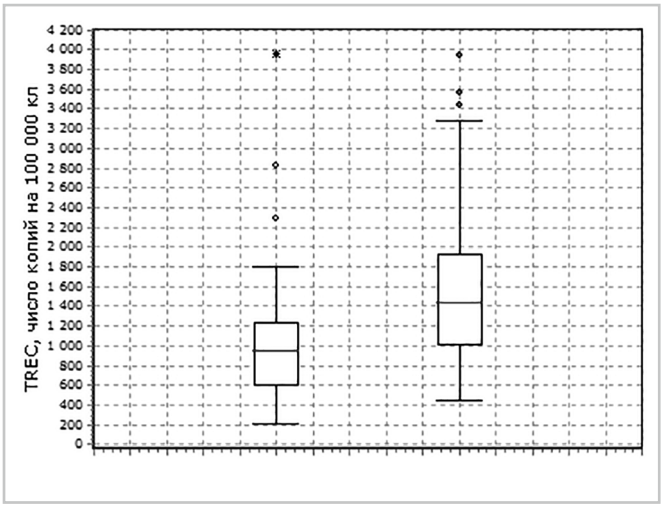

Fig. 2. **KREC indicators in children with congenital heart defects (illustration by the author)**
Рис. 2. **Показатели KREC у детей с врожденными пороками сердца (иллюстрация автора)**

Note: p — differences compared to healthy children (Mann — Whitney test)

Примечание: p — различия по сравнению со здоровыми детьми (критерий Манна — Уитни)

come (Table 1). However, due to the small number of groups, the differences were not statistically significant. As expected, TREC content was significantly lower in infants with lymphopenia (lymphocyte level less than 3000 cells/μL) — 729.0 [567.0; 1332.0] copies per 10^5 cells, p = 0.049.

KREC copy number was lower in newborns with CHD in the genetic disease pattern (1180.5 [573.5; 1579.5] copies per 10^5 cells, p = 0.04) compared to children with CHD without genetic syndromes. No statistically significant difference was obtained depending on the type of CHD, outcome of the disease.

TREC values were found to be decreased in newborns with CHD and infectious complications, 923 [563; 1245] when compared to the group without infectious episodes — 1877 [1427; 2855] copies per 10^5 cells, p = 0.001. Similar patterns were found when analyzing KREC, 708.5

Table 1. **TREC and KREC indicators in newborns with heart defects, depending on demographic characteristics (author's table)**
Таблица 1. **Показатели TREC и KREC у новорожденных с пороками сердца в зависимости от демографической характеристики (таблица автора)**

Indicators	Genetic disease		Lymphocyte count		Nature of CHD		Outcome	
	with n = 8	without n = 35	>3000 kl/μl n = 14	<3000 kl/μl n = 29	with cyanosis n = 5	without cyanosis n = 38	alive n = 39	died n = 4
TREC, copies /10^5 cells	1180,5 [573,5; 1579,5]	1201,0 [702,0; 1800,0]	1233,0 [923,0;1 910,0]	729,0 [567,0; 1332,0]*	1201,0 [702,0; 1483,0]	1359,0 [694,0; 1800,0]	1198,0 [702,0; 1800,0]	959,5 [520; 1891,5]
KREC, copies /10^5 cells	533,5 [417,0; 792,5]**	1020,0 [632,0; 1451,0]	813,5 [585,0; 1514,0]	1020,0 [619,0; 1245,0]	954,0 [798,0; 1260,0]	944,0 [563,0; 1245,0]	943,0 [792,5; 2507,5]	954,0 [563,0; 1260,0]

Note: * — the reliability of differences in indicators depending on the number of lymphocytes, ** — the reliability of differences in indicators in children with and without genetic diseases, p < 0.05 (Mann — Whitney criterion)

Примечание: * — достоверность различий показателей в зависимости от числа лимфоцитов, ** — достоверность различий показателей у детей с генетическими заболеваниями и без них, p < 0,05 (критерий Манна — Уитни)

[430.3; 1047.8] and 1260 [954; 1747] copies per 10^5 cells, respectively, p = 0.01. By ROC analysis, a decrease in TREC of less than 650 copies per 10^5 cells was able to predict infectious complications in newborns with CHD (OR, 10.1; CI 2.35-43.3, p = 0.049) with sensitivity (87.4%), specificity (64.7%), positive (78.7%) and negative (73.3%) predictive value (AUC, 0.87).

A decrease in KREC less than 580 copies per 10^5 cells increased the risk of infectious complications in neonates with CHD (OR, 10.9; CI 2.34-51.2, p = 0.067), but the level of statistical significance was higher than 0.05.

DISCUSSION. Despite modern achievements in the therapy of congenital heart disease, children with CHD have a high risk of infectious morbidity and mortality during the newborn period [9, 12, 13]. It has been determined that structural CHD is accompanied by a decrease in the number and maturity of immune cells, decreased differentiation of T-lymphocytes in the thymus, IgG and IgA deficiency [14, 15]. Such features of immune status determine the increased incidence of RS-infection, increasing the risk of bronchopneumonia and bronchiolitis with lethal outcome at least 25 times [15].

It has been shown that CHD predispose to the development of sepsis and prolonged ALV, which is due not only to changes in the small circulation but also to immune compromise [16]. Routine thymectomy in the correction of CHD may exacerbate T-cell lymphopenia and sensitivity to infections subsequently [17, 18].

In the present study, newborns with CHD were found to have lower levels of TREC and KREC compared to healthy infants in our cohort, as well as when compared to reference values obtained previously using reported test systems [19].

Abnormal TREC indicators were detected in 2 (4.7 %) children who did not have molecular genetic features of primary IDS. Their share in the structure of secondary lymphopenia according to the results of NS in the region amounted to 22.2%.

A pattern of decreased TREC in patients with low absolute lymphocyte counts was obtained.

According to other research centers, the share of congenital heart defects in the structure of secondary lymphopenia ranges from 24 to 40% [1, 7, 20]. In the work by K. Kennedy et al. abnormal TREC levels (below the threshold values defined for PID) were determined in 0.99% of newborns with CHD [21].

In 9 children with CHD with a verified genetic defect (Down, Edwards, Aper syndromes), a reduced KREC copy number was found compared to infants with CHD without genetic syndromes. At least 13 diseases combining heart defects and genetically determined immunodeficiency are known, including Down syndromes [22, 23], Di Giorgi [24, 25, 26], Kabuki [27, 28], Turner [15], congenital asplenia [29]. TREC and KREC have been found to be decreased in these diseases [22, 24].

Some of the genes (CHD7, FOXN-1, GATA4, JAG1, NKX2, TBX1) associated with CHD play a key role not only in cardiac morphogenesis but also in thymus development [30]. Thus, haplonephrectomy of TBX1, a key gene in Di Giorgi disease, contributes to the development of heart defects, hypoplasia of the thymus and parathyroid glands, controlling the expression of almost 2000 genes [31, 32].

Most patients with Down syndrome have a small thymus with an abnormal structure [22]. Altered myelo- and lymphopoiesis causes a decrease in T- and B-cells in 90% of children [34]. Changes in microRNA expression in immune cells, increased signaling from TLR2 causing immune dysregulation are considered as potential mechanisms for the development of IDS [34]. Low TREC copy number in newborns with Down syndrome reflects a decrease in the differentiation of T-lymphocytes in the thy-

mus and their transport into the peripheral bloodstream [22].

Fundamental studies of recent years have been devoted to the study of the relationship between congenital heart defects and immune defects [9, 15, 35]. It has been shown that the embryonic development of thymus and heart are united by common precursor cells — neural crest cells involved in the development of the cardiovascular system, as well as the thymus capsule and pericytes [35]. Loss or dysregulation of neural crest cell signaling pathways contributes to both heart defects and thymic hypoplasia [35, 36].

In addition, perinatal risk factors such as medications, cigarette smoking, gestational diabetes mellitus, malnutrition or congenital infections are associated not only with the development of heart defects but also with intrauterine thymus atrophy [37, 38]. The defect in T-lymphocyte development in neonates with CHD is reflected in the peripheral compartment of immunocompetent cells [9]. Patients with heart defects and thymus atrophy have low numbers of T-lymphocytes, as well as naive T cells (RTE) that have recently completed intrathymic development and migrated into the peripheral bloodstream [9]. Low RTE counts correlate with reduced TREC levels [5, 39, 40].

In the study by B.T. Davey et al. study, the median TREC level in newborns with CHD was significantly lower than in the population [14]. Patients with CHD requiring hospitalization for an infectious disease had lower TREC values compared to children without infectious complications [14], which is consistent with our findings.

Low TREC values have been found in neonates with severe critical heart disease [35, 41, 42]. It has been determined that abnormal TREC and KREC can be used as predictors of potentially fatal infections in premature infants with CHD [14, 43].

Unlike other studies [41, 42, 44], we did not obtain statistically significant differences depending on the type of CHD and its severity, which may be due to the insufficient number of subjects in the groups with cyanotic heart defects and deceased children.

It should be considered that routine thymectomy early in the course of CHD correction may exacerbate T-cell lymphopenia and immune compromise in infants with low TREC at birth and worsen prognosis [17, 18].

CONCLUSION. Thus, TREC and KREC copy number reduction is observed in neonates with heart defects, which makes it possible to identify patients at high risk of infectious complications.

A reduction in TREC copy number below 650 copies per 10^5 cells in neonatal screening allows identification of children with CHD at high risk of infection, opening potential opportunities for preventive therapy.

Children with CHD and identified secondary lymphopenia may be recommended prophylactic courses of antimicrobial agents, immunoglobulin replacement therapy, use of palivizumab, and thymus preservation during correction of CHD [14].

Limitations of the present study include the retrospective design, small number of subjects, and short follow-up interval.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted taking into account the requirements of the Helsinki Declaration of the World Association "Ethical Principles of Conducting Scientific Medical Research with human Participation" as amended in 2000 and the "Rules of Clinical Practice in the Russian Federation" approved by Order of the Ministry of the Russian Federation dated 06/19/2003, No. 266. This study was approved by the Interdisciplinary Local Ethics Committee of the Stavropol State Medical University of the Ministry of Health of the Russian Federation.

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THE AUTHORS' CONTRIBUTION TO THE WORK

Lyudmila Y. Barycheva — research design and methodology, writing the text.
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Andrey A. Puchkov — project administration, research organization.
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