Severe congenital T-lymphocytopenia may affect the outcome of neonatal intensive care

Ivana Hulinkova[1], Veronika Medova[2], Andrea Soltysova[2,3], Veronika Dobsinska[4], Andrej Ficek[3], Peter Ciznar[1]

Aim. Circular DNA segments TREC (T-cell receptor excision circles) formed during T-lymphocyte maturation in the thymus, are a sensitive marker of thymic lymphocyte production in a broader manner. Quantification using qPCR is proposed as a surrogate marker of T cell malfunction in various primary and secondary conditions in a non-SCID selected risk newborn population.

Methods. We collected 207 dry blood spot samples during the years 2015–2018, from newly admitted risk newborns. TREC values calculated per 10^6 cells were determined and a cut-off values of 5th percentile was set. The positive control group consisted of patients (n=13) with genetically confirmed SCID.

Results. The median TREC value was 34,591.56 (18,074.08–60,228.58) for girls resp. 28,391.20 (13,835.01–51,835.93) per 10^6 cells for boys, P=0.046. Neonates born by C-section have been found to have higher TREC levels compared to neonates born by spontaneous delivery (P=0.018). In the group of preterm newborns (n=104), 3.8% had TREC value < 5^{th} percentile, half of them died due to sepsis as opposed to no fatalities in preterm newborns with sepsis and TREC value > 5^{th} percentile. In the group of term newborns (n=103) 9 children (8.7%) had TREC < 5^{th} percentile, half of them were treated for asphyxia, with no fatal complications.

Conclusion. TREC levels calculated for the 5th percentile of a risk neonatal group is suggested as a surrogate marker for increased risk of fatal septic complication. Early recognition of these newborns within a risk scoring system using TREC levels could lead to potentially lifesaving interventions.

Key words: TREC, SCID, immunodeficiency, newborn screening, risk neonates

Received: February 6, 2023; Revised: June 5, 2023; Accepted: June 14, 2023; Available online: July 10, 2023
https://doi.org/10.5507/bp.2023.028
© 2024 The Authors; https://creativecommons.org/licenses/by/4.0/

[1]*Department of Paediatrics, Faculty of Medicine, Comenius University and National Institute of Children's Diseases, Bratislava, Slovak Republic*
[2]*Institute for Clinical and Translational Research, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, Slovak Republic*
[3]*Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic*
[4]*Department of Paediatric Haematology and Oncology, Bone Marrow Transplantation Unit, Faculty of Medicine, Comenius University and National Institute of Children's Diseases, Bratislava, Slovak Republic*
Corresponding author: Peter Ciznar, e-mail: ciznarpeto@gmail.com

INTRODUCTION

Severe T cell immunodeficiency defined as severe combined immunodeficiency (SCID) syndrome presents within a few weeks after birth with mostly opportunistic viral, yeast, or yeast-like fungi (e.g. Pneumocystis jirovecii) infections affecting the respiratory and gastrointestinal tracts[1]. Timely recognition has been found to be crucial for the clinical outcome of primary, genetically determined immunodeficiencies. In 2005, Chan and Puck described a method for measuring T-cell receptor excision rings (TREC) isolated from dried blood spots (DBS) from newborn screening test (NBS) as a potential approach to identify infants with SCID (ref.[2]).

TRECs are small circular DNA elements formed during the rearrangement of T cell receptor (TCR) genes in the thymus during the process of V(D)J recombination. They are stable and do not duplicate during mitosis, representing recent emigrant T cells from the thymus. Thus, TRECs are a marker of recently generated T cells. Low or undetectable TREC indicates insufficient production of T cells by the thymus[3]. Importantly, the presence of maternal T cells does not cause a spurious rise in TRECs, making them suitable for screening[4]. TRECs can be easily detected from dry blood spots (DBS) on the filter paper of Guthrie cards and measured by quantitative real-time PCR (ref.[5]). SCID screening is already part of the national newborn screening program in the US along with 10 other countries, with various pilot programs underway in a number of others[6]. Although the TREC test is highly sensitive, it is not specific. TRECs serve not only to identify SCID, but can also identify non-SCID conditions with congenital T-cell lymphopenia (TCL). These non-SCID conditions include syndromic causes (DiGeorge syndrome, Noonan syndrome, VACTERL syndrome etc.), T cell loss (intestinal lymphangiectasia, gastroschisis, chylothorax, congenital heart defects), or T cell destruction (congenital HIV, neonatal leukemia). Another reported cause of secondary TCL is prenatal use of immunosuppressive or immunomodulating drugs by the mother[7]. The usefulness of this parameter when used instantly within the neonatal period has been questioned, as the compli-

cations of SCID are mostly seen later. Neonatal T cells have been generally considered to have less potent immune reactivity and early immune evaluation is rather exceptional in newborns in intensive care units. However, it was recently shown that neonatal T cells could provide fast-acting immune protection against various foreign pathogens[1,8]. The aim of this study was to evaluate the effect of TREC copy numbers on the clinical outcome and survival of children hospitalized in a Neonatal Intensive Care Unit (NICU) in a pediatric hospital. The study is based on a previously defined age-specific physiological levels and cut-off values[9]. We emphasize the variability of TREC cut-off values used among different countries and ethnicities, supporting the need for specific population adjusted values[10].

MATERIAL AND METHODS

Patients

We conducted a prospective study by including samples from newborns who were hospitalized at the NICU of a university pediatric hospital from February 2015 to July 2018. The children admitted to this NICU represent those with a variety of congenital defects and inborn errors. The first inclusion criterion was that all newborns were no older than 3–5 days, due to the need for standardized DBS collection, regardless of their gestational age and birth weight. The second inclusion criterion was an informed consent which was signed by the parents. Therefore, children unaccompanied by a parent were excluded. Informed consent was obtained in 98.1% of available newborns. We collected DBS from 207 newborns on WhatmanTM 903 filter paper. Of this number, 123 were boys and 84 were girls, 104 were born prematurely (≤ 36+6 gestational week) and 103 newborns were born at term (≥ 37+0 gestational week). Cohort characteristics are shown in Table 1. DBS samples were collected 3 to 5 days after birth. For each newborn, we collected data such as: gestational age, Apgar score, mode of delivery, birth weight, infectious complications, length of ATB treatment and hospitalization, length of intubation, if applied, and selected laboratory parameters (leukocytes, lymphocytes, inflammatory parameters).

As internal disease controls, we used samples from 13 patients with previously diagnosed SCID (5 patients with ADA deficiency, 6 patients with IL2RG mutation, and 2 patients with Omen syndrome RAG1 mutation).

Table 1. Characteristics of the cohort of 207 newborns hospitalized in the NICU with median and minimum/maximum values.

Monitored variables	Numerical expression
Gender, n	
male/female	123/84
Gestational age, weeks	
median	37 (34–39)
minimal – maximal range	24–42
Birth weight, grams	
median	2920 (2030–3445)
minimal – maximal range	620–4850
Mode of delivery, n	
spontaneous/C-section	96/111
TREC, copies/10^6 cells	
median	29,914.75 (16,009.97–52,710.03)
minimal – maximal range	381.01–35,479942.67
Leukocytes 10^9/L	
[reference range 6–17×10^9/L]	
median	13.66 (10.13–18)
minimal – maximal range	1.85–41.4
Lymphocytes 10^9/L	
[reference range 3–9.5x10^9/L]	
median	3.43 (2.53–4.83)
minimal – maximal range	0.66–8.37
CRP mg/L	
[reference range 0–10 mg/L]	
median	18.9 (3.5–49.8)
minimal – maximal range	0–405
PCT ug/L	
[reference range 0–0.5 ug/L]	
median	3.44 (0.72–11.91)
minimal – maximal range	0–259.14
Length of ATB, days	
median	5 (1–7)
minimal – maximal range	0–87
Length of hospitalization,	
days	16 (12–24)
median	3–90
minimal – maximal range	
Length of intubation, hours	
median	0 (0–93.5)
minimal – maximal range	0–936

DNA extraction and Quantitative PCR Assay

We performed DNA extraction from dry blood spots using Extracta DBS (QuantaBio, USA). The collected 2.0 mm punch was washed in 100 µL of DBS extract buffer and centrifuged for 5 min at 3500 rpm in an Eppendorf MiniSpin. Subsequently, DNA was eluted to a fresh 50 µL of DBS extract buffer by incubation at 95 °C for 30 min. DNA from whole blood samples (500 µL) was isolated with Gentra Puregene Blood Kit (Qiagen, Germany) and eluted to 100 µL of DNA hydration solution, and stored at –20 °C. Real-time PCR from DBS samples was run in final volumes of 10 µL containing 5 µL of PERFECTA QPCR FASTMIX II L-ROX (QuantaBio, USA), 2 µL of DNA isolated from DBS, and 0.3 µM primers and probe. Real-time PCR amplification was performed on the Stratagene Mx3005P Real-time PCR System (Agilent Technologies, USA) with the following program: an initial 15 min denaturation at 95 °C followed by 50 cycles of 30 s at 95 °C denaturation, 30 s at 59 °C annelation, and 30 s at 72 °C polymerization. The quantification of TREC and TRAC (internal control) were based on a standard curve generated from diluted plasmid containing 1 copy of TREC and 1 copy of TRAC. The amplification of TRAC (T-cell receptor alpha chain constant region) was used as an internal control of successful DNA isolation and real-time PCR reaction.

Levels of TREC were calculated as the number of copies per 10^6 cells according to the following formula:

$$TREC\ copies\ /10^6\ cells = \frac{TREC\ Qty}{TRAC\ gene\ Qty\ /\ 2} \times 10^6$$

Cut - off values were given as the 5th percentile and were defined for TREC molecules per 10^6 cells as 5202 for DBS isolation.

Statistical data analysis

Statistical analyses were carried out using OriginPro 2021 v.9.8.0.200. Data distribution was assessed using the Shapiro-Wilk test. Normally distributed data are expressed as means ± SD. Non-normally distributed data are expressed as the median and 25th do 75th percentile. Given the nonparametric distribution of the data, the Mann-Whitney U test was used for group comparison analyses, and the Spearman correlation coefficient was calculated for correlation analyses. Differences were considered statistically significant when the *P* value < 0.05.

RESULTS

We enrolled 207 newborns aged 1–3 days, referred from local maternity hospitals in the region. Due to the specialization of the NICU, most of the children were full-term or slightly premature. Diagnoses at admission were asphyxia 14.5% (30), various congenital defects 38% (78), RDS 24% (50), other neonatal emergencies 23.5% (49) with a more precise distribution shown in Table 2. When diagnosing asphyxia, we followed the guidelines of the American Academy of Pediatrics (AAP): presence of deep metabolic or mixed acidemia (pH < 7.00) in the umbilical cord blood sample, Apgar score at 5 min <3, evidence of multiorgan dysfunction and clinical neurological manifestations.

Effect of gender and birth weight on TREC values

By analyzing the relationship between gender and TREC levels, regardless of gestational age, we found a statistically significantly lower TREC (*P*=0.046) in boys (n=123) compared to girls (n=84) (Fig. 1A). The median TREC for girls was 34,591.56 (18,074.08–60,228.58) and for boys 28,391.20 (13,835.01–51,835.93).

Throughout pregnancy, the fetal thymus undergoes a process of growth and differentiation. Thus, birth weight

Table 2. Spectrum of diagnoses of newborns at the time of NICU admission.

Diagnosis	Percentage of patients	Number of patients
Asphyxia	**14.5%**	**30**
Congenital defects	**38%**	**78**
Congenital defects of gastrointestinal tract	19 %	40
Congenital heart defects	3%	5
CNS congenital anomalies	11%	22
Congenital anomalies of the kidney and urinary tract	5%	11
RDS	**24%**	**50**
Others	**23.5%**	**49**
AB0 and Rh isoimmunization	2%	4
Intolerance oral intake and hypotrophy	5%	10
Adnate infection	6.5%	14
Genetic syndromes	1%	2
Inborn errors of metabolism	1%	2

Table 3. Distribution of the set of newborns by gestational age and evaluation of the median, minimum and maximum number of TREC copies.

Group of patients	Median TREC 10^6 cells (25^{th}–75^{th} perc.)	Min. TREC 10^6 cells	Max. TREC 10^6 cells
Preterm, n=104	32,780.67 (18,287.63–53,017.29)	3291.92	35479942.67
Extremely preterm (< 28. weeks), n=6	10,223.21 (5517.24–33,284.88)	3291.92	65,161.96
Very preterm (28.–31. weeks), n=18	29,525.35 (21,171.11–50,135.8)	6095.02	35479942.6
Moderate to late preterm (32.–36. weeks) n=80	34,098.15 (20,099.64–53,421.61)	3470.7	5911973.00
Full-term (37.–41. weeks), n=103	24,999.88 (11,996.65–49,167.97)	381.01	13387668.03

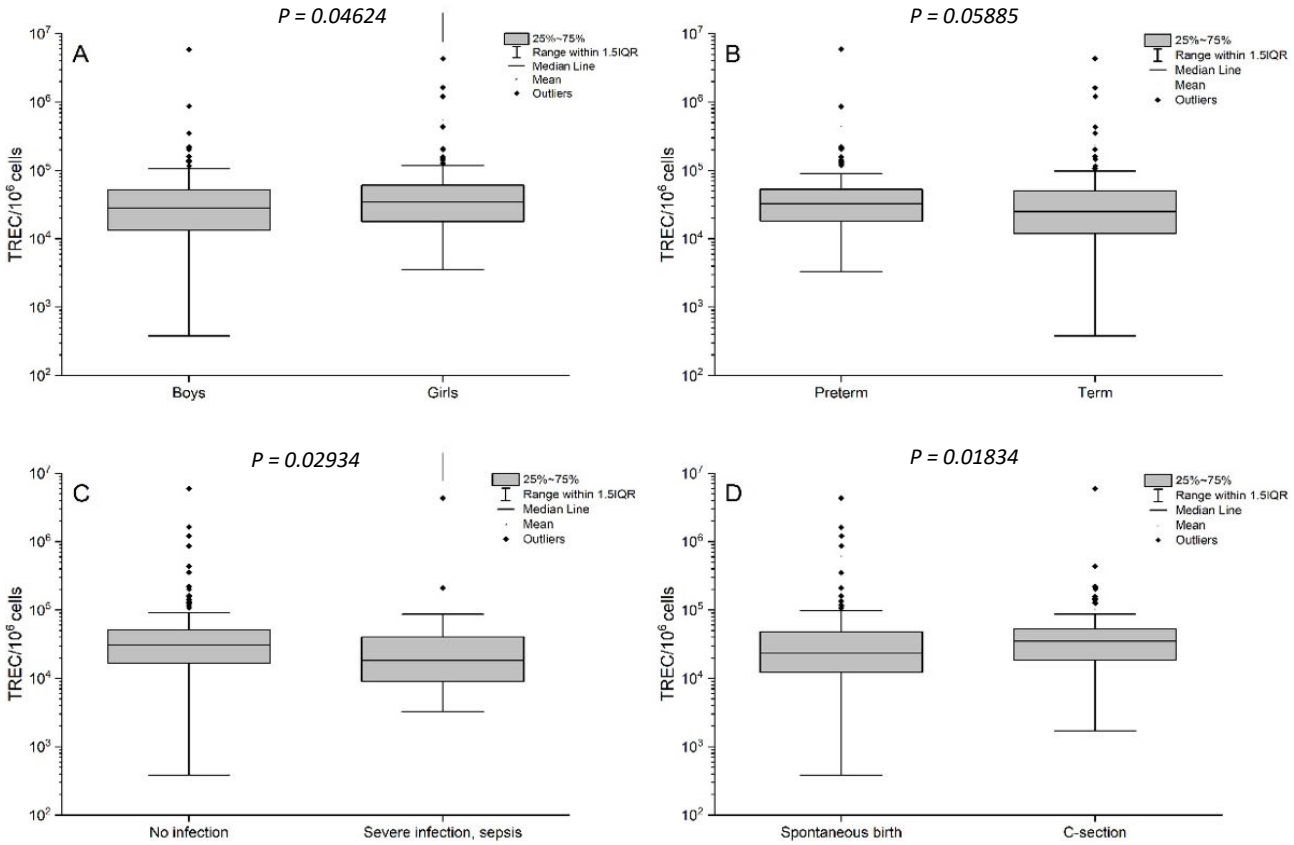

Fig. 1. TREC levels by gender (A), gestational age (B), infectious complication (C) and mode of delivery (D).

alone could potentially influence TREC levels. However, by correlating TREC levels and birth weight in our selected cohort, there was no statistical association (Fig. 2A).

Comparison of TREC levels with respect to gestational age and mode of delivery

Table 3 shows the distribution of the patient cohort according to gestational age. We compared TREC levels in premature and full-term neonates (Fig. 1B). We did not find significantly lower levels of TREC in premature newborns compared to full-term newborns P=0.058. In our cohort, there were 104 preterm infants with a median TREC of 32,780.67 (18,287.63−53,017.29) and 103 full-term infants with a median TREC of 24,999.88 (11,996.65−49,167.97). Correlation analysis also did not identify a relationship between the increase in TREC with increasing gestational age (Fig. 2B). Gestational age is largely related to the mode of delivery. In our group, 96 newborns were born by spontaneous delivery and 111 by cesarean section. Through analysis, we found that neonates born by spontaneous labor had lower TREC levels compared to neonates born by cesarean section with statistical significance, P=0.018 (Fig. 1D).

TREC values, septic complications and length of ATB treatment

In our patient cohort, we aimed to compare TREC levels in patients with sepsis (n=40) and those without any infectious complications (n=128). We define neonatal sepsis as a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life. We followed the criteria of the AIIMS Protocols in Neonatology, 2015 (Table 4) (ref.[11]). A statistically significant association was found between TREC values and the incidence of sepsis, P=0.029 (Fig. 1C). We showed that patients with lower TRECs are more often affected by serious infections. We found no significant relationship between low TREC and the length of ATB treatment used or intubation either, P>0.05. Of laboratory parameters, we analyzed the relationship of the concentration of the absolute number of lymphocytes and the TREC level and found no positive correlation between these two parameters (Fig. 2C).

TREC below the set cut-off value

On the basis of a predetermined cut-off value[9] according to the same TREC quantification method, we evaluated a group of patients with TREC levels in the so-called gray zone. Cut-off values were reported as the 5th percentile and were defined for TREC molecules per 10^6 cells as 5202 copies for isolation from DBS. TRECs were independent on total lymphocyte count as showed by Fig. 2C.

In the preterm group (n=104), 4 children had a TREC value < 5th percentile. Their clinical presentation was dominated by sepsis. Two were born by cesarean section and

two by spontaneous birth, all were males. In this group of premature patients, gestational week of birth was 27th (P1), 28th (P2) and 36th (P3, P4), respectively. Similarly, was the birth weight (P1=1200 g, P2=1250 g, P3=3000 g, P4=3550 g). Half of these patients (P1, P2) died. In the preterm newborns with TREC > 5th percentile (n=100), 15 had sepsis and none of them had died. We found a statistically significantly higher (P<0.01) incidence of sepsis in preterm newborns with TREC < 5th percentile. Mortality rate was 50% of preterm newborns with sepsis and TREC < 5th percentile, compared to no fatal complication in preterm newborns with sepsis and TREC > 5th percentile.

In the full-term newborns (n=103), 9 children had TREC < 5th percentile. Half of newborns in this group had neonatal asphyxia, but no fatal complication occurred. Seven newborns were born by spontaneous delivery and 2 by cesarean section. There was also a predominance of boys in this group, only one newborn was female. None of the DBS samples in our neonatal cohort showed undetectable TRECs. All samples in the group of confirmed SCID cases had zero TREC levels.

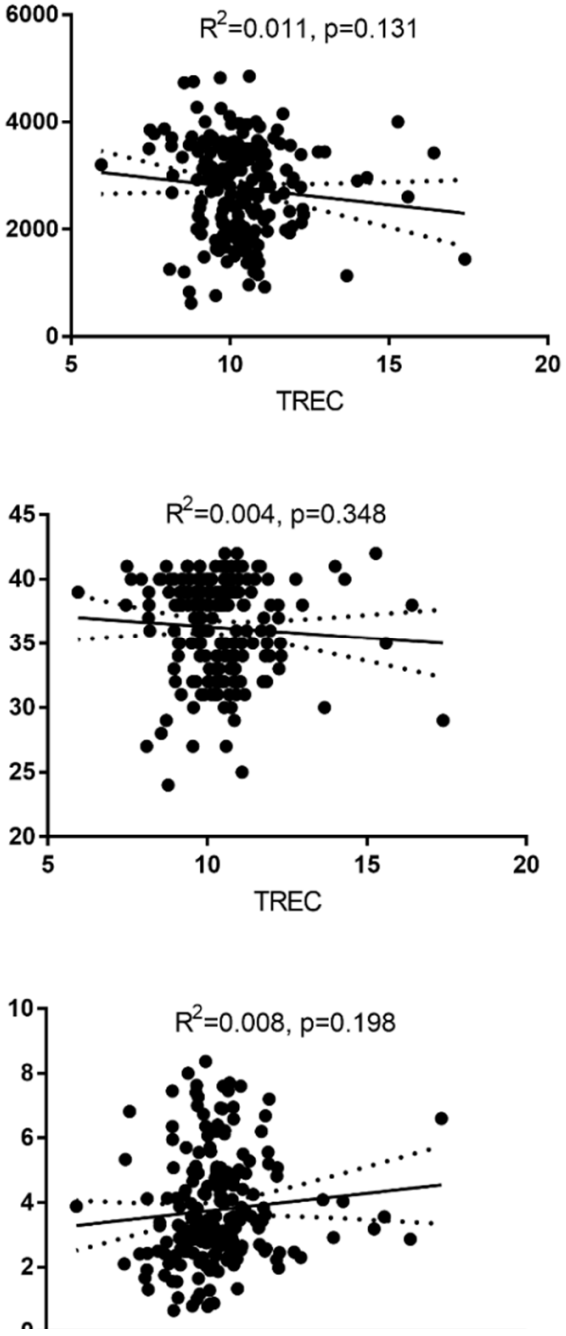

Fig. 2. Correlation between TREC birth weight (A), gestational age (B) and lymphocyte count (C).

DISCUSSION

This is the first study where the TREC method was not primarily used to detect SCID patients, but rather identify neonates with low T cell function due to non-SCID conditions. As an example, low TREC levels may be due to immunosuppressive treatment of the mother or prenatal environmental immunosuppression causing exposures[12]. An increasing number of countries, including Slovakia, are introducing nationwide newborn screening programs for SCID using the TREC method as a standard. However, to achieve optimal sensitivity and specificity, the cut-off levels for a positive SCID screening are set quite low. Thanks to this approach an important group of children with very low T lymphocyte function will be captured by the National Screening Program. Another clinically relevant proportion will remain in the grey zone, above the detection limit. Using a 5-percentile limit allowed us to identify patients with milder phenotypes and lower, but detectable TRECs, in a focused specific population adjusted for age. In the past, during the neonatal period a child was presumed to be fully protected by maternal antibodies and T lymphocyte functions were characterized as very immature, clinically insignificant. More recent findings have shown that T lymphocytes are reactive, playing an important role in our adaptation to the outer environment by rapid development into effector or regulatory cells[1]. Hence TREC could be used as a surrogate marker for identification of T lymphocyte malfunction and incorporated into a risk scoring system. For the diagnosis of an immunodeficiency in general, blood analysis including immunophenotyping of lymphocyte subpopulations is a preferred and well-established method. In the framework of newborn screening, TREC analysis has come to the fore in recent years. It is a fast, sensitive and inexpensive tool for screening and diagnosing severe forms of lymphopenia. Its advantage lies mainly in the possibility of using small volumes of blood for DNA extraction which is often problematic in pediatric populations. However, greater attention should be paid to the variability of protocols for the analysis of TREC levels in different laboratories. In addition to the methodology used, genetic and ethnic differences between populations also play a role.

The main objective of the study was to determine the relationship of TREC copy number in the neonatal period on different variables and to determine possible risk factors that can be derived from TREC copy number. In our cohort we reported a significant difference in TREC levels between boys and girls in favor of girls. Correlated with

Table 4. Clinical and laboratory criteria of neonatal sepsis (the presence of at least 2 clinical and 2 laboratory signs) (ref.[11]).

Clinical criteria
body temperature above 38.5 °C or below 36 °C
tachycardia – heart rate above 2SD with respect to age without external stimulus, bradycardia for the given age
tachypnea – above 2 SD due to age or the need for artificial pulmonary ventilation
cardiovascular dysfunction
Laboratory criteria
number of leukocytes < $4x10^9$/L or > $20x10^9$/L
IT index > 0.2
platelet count < 100 x 10^9
CRP > 15 mg/L or procalcitonin > 2 ng/mL
Hyperglycemia > 10 mmol/L or hypoglycemia < 2.5 mmol/L
Metabolic acidosis: BE < -10 mmol/L or lactate > 2 mmol/L

our results are studies indicating a higher level of TREC in the blood of women[13] and an association between low TREC and male gender of infants[14]. Similarly, male gender was identified as a risk factor for low postnatal TRECs by Lisse et al. who found that the umbilical cord blood of girls contains a higher number of CD4+ T-lymphocytes, a higher ratio of CD4/CD8 T-lymphocytes. and lower CD8+ T-lymphocyte and NK cell counts than cord blood from male infants[15].

TREC levels tend to decrease with increasing age. The number of TREC copies in the blood samples of older children and adults is 10-fold and 100-fold lower than in healthy newborns, which is related to a decrease in thymus function with increasing age[16]. Regarding the neonatal period, preterm newborns have functional deficiencies in their immune system and are more susceptible to infections[17].This fact is supported by studies showing a correlation between prematurity and low TREC levels[14,18]. According to these studies, TREC usually normalizes with increasing gestational age. Surprisingly in our group, we found no statistically significant difference (P=0.058) between the TREC levels in premature and full-term newborns. The most likely reason was that the majority of the cohort comprised of term children with a median of gestational age 37 week.

In analyzing the mode of delivery in our patient cohort, we found a significant correlation (P=0.018) between low TREC levels and neonates born by spontaneous delivery. We assume that the given results are related to incomplete information about the management of spontaneous labor (prolonged, complicated, instrumental etc.). Cord blood cortisol levels have been reported to be highest in forceps-born infants, and lowest in cesarean section[19]. Release of stress hormones, especially cortisol, can negatively affect TREC levels[20]. In general, childbirth is associated with an acute recruitment of leukocytes[21]. In connection with the stress of childbirth, there is a significant increase in the total number of white blood cells in the umbilical cord blood, as well as an increase in the level of IL-8 (ref.[22]). It can be assumed childbirth stress can act as a regulator of the immune system of newborns[19]. In contrast, in a large study by Schlinzing et al. in a study population of 6014 neonates born between 35–42 weeks of gestation, low TREC levels were noted more often after elective cesarean section[14]. However, Gul et al. did not find the method of delivery affected TREC values[23]. From these different results of the effect of mode of delivery on TREC levels, it is important to point out that we probably need more information about prenatal and perinatal events for all studied populations.

Regarding the relationship between low birth weight and low TREC levels, several studies have been published to date[12,14,24,25]. A positive correlation between TREC levels and birth weight was revealed in most[26]. However, in one study, similar to ours, the authors found that newborns born with low birth weight (< 2500 g) had significantly higher TREC concentrations in peripheral blood cells than those born with normal birth weight ≥ 2500 g (ref.[27]). In that study, children of the low birth weight group had significantly lower interleukin 7 plasma concentrations and a lower percentage of CD3 T cells. Higher TREC values are attributed to greater turnover of peripheral T cells (lower concentration of CD3+ cells) due to immune activation, resulting in a greater need for replenishment from the thymus and increasing T cell receptor excision circles.

Early detection of severe T-lymphopenia enables early diagnosis and thus ensures the possibility of early application of appropriate therapy for SCID (HSCT, gene and enzyme therapy) even before the appearance of serious clinical symptoms, which will not only improve the quality of life of patients, save their lives, but also reduce financial and economic costs for treatment[28-30]. In addition to SCID, other forms of immune deficiency could be detected. Therefore, positive samples must be followed by appropriate second-stage testing to help define the disease more precisely. Confirmation by flow cytometry is recommended for full-term neonates with an out-of-range TREC result or for any neonate with zero TREC values at newborn screening[31].

Our study identified TREC quantity in a risk group. We found a significant association between low TREC values and the incidence of sepsis in general (P=0.029). We evaluated a group of patients with low TREC levels

within a septic group of patients. In preterm infants, all with TREC below the 5th percentile had a clinical picture of sepsis, and half of these patients died. We acknowledge that the deceased newborns belong to the extremely/very preterm group, however only targeted larger studies could truly evaluate this effect. We identified an association between the mortality of preterm neonates with sepsis in patients with TREC < 5th percentile (50% of neonates died) compared to patients with normal TREC levels. We therefore assume that a low TREC level may be a surrogate marker for a fatal outcome in preterm neonates. Hypothetically, monitoring TREC parameters could allow us to make additional clinical interventions (immunoglobulin administration, anti-infectious measures). We are aware of the limitations like the limited size of the tested and selected population at the NICU of a pediatric university hospital. Possible genetic and ethnic differences should be taken into account as well. However as mentioned above this is the first pilot study in which the TREC method is primarily used to identify neonates with low T cell function due to non-SCID conditions and to assess the possibility of using TREC as a biomarker.

TRECS LIMITATIONS

Alongside its advantages, the TREC method has certain pitfalls and limitations. We can find a wide range of cut-off values in different studies because different algorithms are used at different screening sites. The cut-off values differ according to the size of the punch used, the method of extraction, the use of the control gene and the methodology itself[32]. Guthries card punch size for example used in the UK was 1.5 mm in contrast to the standard 3.2 mm suggested by the EnLite neonatal TREC kit (Perkin Elmer) (ref.[33]). TREC values could be expressed per volume or a conversion to cell count can be used. The latter method of measurement is considered more accurate.

CONCLUSION

Early recognition of a T lymphocyte deficiency, whatever the cause is, could affect clinical complications or even life-threatening events. Quantitative assessment of TREC as a surrogate marker of T lymphocyte function is not only an excellent screening tool for severe combined immunodeficiency syndrome, but is proposed as a simple parameter for risk neonates. Patients with lowest levels, although over the cut-off for NBS, appear to have more frequent infectious complications and lower survival rate. We definitely need additional studies to confirm whether alone, or in combination with other risk factors, as part of a scoring system, could lead to clinical interventions. Repeated monitoring of TREC levels within a period of time could improve its suitability and evaluate T-cell function reconstitution. A deeper insight into the establishment of normal immune function at birth and its relationship to various perinatal factors remains a challenge.

ABBREVIATIONS

DBS, Dry blood spots; HSCT Hematopoietic stem cell transplantation; NBS, Newborn screening; NICU, Neonatal Intensive Care Unit; SCID Severe combined immunodeficiency; TCL, T-cell lymphopenia; TREC, T-cell receptor excision circles.

Acknowledgements: The authors would like to thank Dr. D. Dolníková and prof. Dr. I. Brucknerova, the leads of NICU for strong support and cooperation to reach out informed consents and DBS samples. The work was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sports of the Slovak Republic and the Slovak Academy of Sciences (VEGA 1/0190/19). This work is generated within the European Reference Network for rare immunological disorders (ERN-RITA). Financial support was provided partially by the Jeffrey Modell Foundation grant.

Author contributions: IH: main author, writing, conception and design of the work, literature search, data collection, data analysis; VM: laboratory analysis, data analysis, figures; AS, AF: data analysis; VD: data collection; PC: consultant, text editor, correction and critical evaluation of the clinical portion of the manuscript.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

REFERENCES

1. Rudd BD. Neonatal T cells: A reinterpretation. Annu Rev Immunol 2020;38:229-47.
2. Chan K, Puck JM. Development of population-based newborn screening of severe combined immunodeficiency. J Allergy Clin Immunol 2005;115:391-8.
3. Kwan A, Puck JM. History and current status of newborn screening for severe combined immunodeficiency. Semin Perinatol 2015;39:194-205.
4. Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, Polis MA, Haase AT, Feinberg MB, Sullivan JL, Jamieson BD, Zack JA, Picker LJ, Koup RA. Changes in thymic function with age and during the treatment of HIV infection. Nature 1998;396(6712):690-5.
5. Borte S, von Döbeln U, Fasth A, Wang N, Janzi M, Winiarski J, Sack U, Pan-Hammarström Q, Borte M, Hammarström L. Neonatal screening of severe primary immunodeficiency diseases using high-throughput triplex real-time PCR. Blood 2012;15(119):2552-5.
6. Quinn J, Orange JS, Modell V, Modell F. Case of severe combined immunodeficiency (SCID) and lymphopenia T cell newborn screening: saving lives...one at a time. Immunol. Res 2020;68(1):48-53.
7. Kuo CY, Garcia-Lloret MI, Slev P, Bohnsack JF, Chen K. Profound T-cell lymphopenia associated with prenatal exposure to purine antagonists detected by neonatal TREC screening. J Allergy Clin Immunol Pract 2017;5(1):198-200.
8. Basha S, Surendran N, Pichichero M. Immune responses in neonates. Expert Rev Clin Immunol 2014;10(9):1171-84.
9. Medova V, Hulinkova I, Laiferova N, Urdova V, Ciznar P, Dolnikova D, Krasnanova V, Fabri O, Ficek A, Soltysova A. The importance of defining the age-specific TREC/KREC levels for detection of various inborn errors of immunity in pediatric and adult patients. Clin Immunol 2022;245:109155.
10. Puck JM. A case of newborn screening for severe combined immunodeficiency and related disorders. Ann N Y Acad Sci 2011;1246:108-17.
11. Agarwal R, Deorari A. AIIMS Protocols in Neonatology, 1st Ed. India: CBS Publishers; 2015.

12. de Felipe B, Olbrich P, Lucenas JM, Delgado-Pecellin C, Pavon-Delgado A, Marquez J, Salamanca C, Soler-Palacin P, Gonzalez-Granado LI, Antolin LF, Borte S, Neth O. Prospective newborn screening for Severe T- and B-lymphocyte deficiency in Seville. Pediatr Allergy Immunol 2016;27(1):70-7.
13. Shakerian L, Pourpak Z, Shamlou S, Domsgen E, Kazemnejad A, Dalili H, Nourizadeh M. Determining Laboratory Reference Values of TREC and KREC in Different Age Groups of Iranian Healthy Individuals. Iran J Allergy Asthma Immunol 2019;18(2):143-52.
14. Schlinzig T, Johansson S, Stephansson O, Hammarström L, Zetterström RH, von Döbeln U, Cnattingius S, Norman M. The rise in immune cell formation at birth varies by mode of delivery and infant characteristics - a population-based cohort study. PLoS One 2017;12(9):e0184748.
15. Lisse IM, Aaby P, Whittle H, Jensen H, Engelmann M, Christensen LB. T-lymphocyte subsets in West African children: influence of age, sex, and season. J Pediatrics 1997;130(1):77-85.
16. Sempowski GD, Gooding ME, Liao HX, Le PT, Haynes BF. T cell receptor excision circle assessment of thymopoiesis in aging mice. Mol Immunol 2002;38(11):841-8.
17. Sharma AA, Jen R, Butler A, Lavoie PM. The developing human preterm neonatal immune system: rationale for further research in this area. Clinical immunology 2012;145(1):61-8.
18. Vogel BH, Bonagura V, Weinberg GA, Ballow M, Isabelle J, DiAntonio L, Parker A, Young A, Cunningham-Rundles C, Fong CT, Celestin J, Lehman H, Rubinstein A, Siegel S, Weiner L, Saavedra-Matiz C, Kay DM, Caggana M. Newborn screening for SCID in New York State: experience from the first two years. J Clin Immunol 2014;34(3):289-303.
19. Gitau R, Menson E, Pickles V, Fisk NM, Glover V, MacLachlan N. Umbilical cortisol levels as an indicator of the fetal stress response to assisted vaginal delivery. Eur J Obstet Gynecol Reprod Biol 2001;98(1):14-17.
20. Prieto-Hinojosa A, Knight A, Compton C, Gleeson M, Travers PJ. Reduced thymus output in elite athletes. Brain Behav Immun 2014;39:75-9.
21. Koenig JM, Stegner JJ, Schmeck AC, Saxonhouse MA, Kenigsberg LE. Neonatal neutrophils with prolonged survival show an increased inflammatory and cytotoxic response. Pediatric Res 2005;57(3):424-9.
22. Yektaei-Karin E, Moshfegh A, Lundahl J, Berggren V, Hansson LO, Marchini G. Birth stress increases in vitro spontaneous and IL-8-induced neutrophil chemotaxis in the human newborn. Children's allergy and immunology 2007;18(8):643-51.
23. Gul KA, Strand J, Pettersen RD, Brun H, Abrahamsen TG. T-cell Receptor Excision Circles in Newborns with Heart Defects. Pediatr Cardiol 2020;41(4):809-15.
24. Pawłowski B, Nowak J, Borkowska B, Augustyniak D, Drulis-Kawa Z. Body height and immune efficacy: testing body stature as a signal of biological quality. Proc Biol Sci 2017;284(1859):20171372.
25. Olbrich P, de Felipe B, Delgado-Pecellin C, Rodero R, Rojas P, Aguayo J, Marquez J, Casanovas J, Sánchez B, Lucena JM, Ybot-Gonzalez P, Borte S, Neth O. A first pilot study on the neonatal screening of primary immunodeficiencies in Spain: TRECS and KRECS identify severe T- and B-cell lymphopenia. An Pediatr (Barc) 2014;81(5):310-17.
26. Ye P, Kirschner DE. Measuring emigration of human thymocytes by T-cell receptor excision circles. Crit Rev Immunol 2002;22(5-6):483-97.
27. Raqib R, Alam DS, Sarker P, Ahmad SM, Ara G, Yunus M, Moore SE, Fuchs G. Low birth weight is associated with altered immune function in children in rural Bangladesh: a birth cohort study. Am J Clin Nutr 2007;85(3):845-52.
28. Castagnoli R, Delmonte OM, Calzoni E, Notarangelo LD. Hematopoietic Stem Cell Transplantation in Primary Immunodeficiency Diseases: Current Status and Future Perspectives. Front Pediatr 2019;7:295.
29. Gaspar HB, Cooray S, Gilmour KC, Parsley KL, Adams S, Howe SJ, Al Ghonaium A, Bayford J, Brown L, Davies EG, Kinnon C, Thrasher AJ. Long-term persistence of the polyclonal T cell repertoire after gene therapy for X-linked severe combined immunodeficiency. Sci Transl Med 2011;3(97):97ra79. doi: 10.1126/scitranslmed.3002715
30. Brown L, Xu-Bayford J, Allwood Z, Slatter M, Cant A, Davies EG, Veys P, Gennery AR, Gaspar HB. Neonatal diagnosis of severe combined immunodeficiency results in significantly improved survival outcome: the case for newborn screening. Blood 2011;117(11):3243-6.
31. Amatuni GS, Sciortino S, Currier RJ, Naides SJ, Church JA, Puck JM. Reference intervals for lymphocyte subsets in premature and full-term neonates without immune defects. J. Allergy Clin Immunol 2019;144(6):1674-83.
32. van der Spek J, Groenwold RH, van der Burg M, van Montfrans JM. TREC-based neonatal screening for severe combined immunodeficiency: a systematic review. J Clin Immunol 2015;35(4):416-30.
33. Adams SP, Rashid S, Premachandra T, Harvey K, Ifederu A, Wilson MC, Gaspar HB. Screening for neonatal dry blood spots in the United Kingdom using the TREC duplex screening test. J Clin Immunol 2014;34(3):323-30.