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RESEARCH

Newborn Screening for Severe T and B Cell Lymphopenia Using TREC/KREC Detection: A Large-Scale Pilot Study of 202,908 Newborns

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Abstract

Newborn screening (NBS) for severe inborn errors of immunity (IEI), affecting T lymphocytes, and implementing measurements of T cell receptor excision circles (TREC) has been shown to be effective in early diagnosis and improved prognosis of patients with these genetic disorders. Few studies conducted on smaller groups of newborns report results of NBS that also include measurement of kappa-deleting recombination excision circles (KREC) for IEI affecting B lymphocytes. A pilot NBS study utilizing TREC/KREC detection was conducted on 202,908 infants born in 8 regions of Russia over a 14-month period. One hundred thirty-four newborns (0.66‰) were NBS positive after the first test and subsequent retest, 41% of whom were born preterm. After lymphocyte subsets were assessed via flow cytometry, samples of 18 infants (0.09‰) were sent for whole exome sequencing. Confirmed genetic defects were consistent with autosomal recessive agammaglobulinemia in 1/18, severe combined immunodeficiency – in 7/18, 22q11.2DS syndrome – in 4/18, combined immunodeficiency – in 1/18 and trisomy 21 syndrome – in 1/18. Two patients in whom no genetic defect was found met criteria of (severe) combined immunodeficiency with syndromic features. Three patients appeared to have transient lymphopenia. Our findings demonstrate the value of implementing combined TREC/KREC NBS screening and inform the development of policies and guidelines for its integration into routine newborn screening programs.

Keywords Inborn errors of immunity · newborn screening · TREC · KREC · severe combined immunodeficiency · agammaglobulinemia

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Introduction

Inborn errors of immunity (IEI) are a heterogeneous group of potentially fatal genetic disorders affecting at least 1 in 10,000 people [1]. Currently, more than 485 forms of IEI are recognized [2]. Severe combined immunodeficiency (SCID) and agammaglobulinemia (AGG) are two of the most well-defined of these conditions, with clear-cut treatment approaches and good prognosis upon early diagnosis (reviewed in [3, 4]). SCID is the most severe form of IEI and is universally lethal within the first 1–2 years of life without early intervention such as hematopoietic stem cell transplantation, gene therapy or enzyme replacement therapy, depending on the underlying genetic defect [5]. Therapeutic success is largely dependent on the absence of active infections and the patient's age. Infants who were transplanted before the age of 3.5 months had significantly better outcomes than those treated later in life (94% survival versus 50%) [6].

An assay for the newborn screening (NBS) for severe combined immunodeficiency was developed in 2005 by Chan and Puck who first described DNA-based testing of T-cell receptor excision circles (TREC) for SCID and other forms of T-cell lymphopenia [7]. SCID screening was first implemented in Wisconsin (USA) in 2008 and now is being used successfully throughout the United States [8, 9]. In Europe, SCID screening was first included in the newborn screening programs in the region of Catalonia in Spain [10], and Iceland in 2017 [11]. Also, newborn screening for SCID is being successfully used in several countries such as Poland, Norway, Denmark, Sweden, Switzerland, Italy, Israel, New Zealand, Norway, and Taiwan, in some regions in Australia as well as numerous provinces in Canada [12]. Therefore, an extensive experience of screening for T lymphopenia and follow-up procedures exists worldwide though exact protocols vary by the country. TREC are small circular pieces of DNA produced by rearrangement of the T cell receptor (TCR) α gene and, thus, a marker of naïve T cell production [13]. Therefore, while some countries utilize purely genetic approach to IEI confirmation suspected by NBS, most protocols call for confirmatory lymphocyte immunophenotyping, including detection of naïve T lymphocyte subsets. Besides early detection of SCID and some other forms IEI accompanied by abnormal T cell production [14] introduction of TREC assays in NBS allowed to calculate the real incidence of SCID in respective countries [15] as well as describe T lymphocyte abnormalities in conditions different from classic IEIs [16].

Shortly after TREC assay description, kappa-deleting recombination excision circle (KREC), circular double-stranded DNA produced during B cell receptor rearrangement, was proposed for a combined TREC and KREC screening approach for severe forms of T- and/or B-cells deficiencies, including different forms of agammaglobulinemia [17]. Yet, introduction of KREC measurements in the national NBS programs has been controversial [18] due to reportedly high false-positive rates [19] and concerns about cost-effectiveness of screening for B cell immunodeficiencies. In opposite to SCID, patients with agammaglobulinemia usually do not have symptoms before 6 months of life and are frequently asymptomatic for several years. Therefore, though KREC assay as part of NBS has been reported in several countries, the experience is limited to smaller/pilot studies. Yet, late start of immunoglobulin substitution leads to severe morbidity and mortality in patients with humoral immunodeficiencies [20], therefore the early diagnosis in this cohort is quite important.

Here we present the results of the pilot NBS screening study, implementing TREC/KREC detection, performed in Russia on more than 200,000 newborns.

Materials and Methods

NBS Pilot Program

TREC/KREC-based pilot screening was conducted from January 1, 2022 to February 19, 2023 in eight regions of the Russian Federation (Krasnodar Territory, Vladimir, Orenburg, Ryazan, Sverdlovsk Regions, Republic of Bashkortostan, Republic of North Ossetia-Alania, Chechen Republic).

The total number of screened neonates was 202,908. Written parental informed consent was obtained for all newborns who participated in the NBS program. All samples were anonymized using a 10-digit CODE128 barcode on the Guthrie cards. For each newborn, the following information was collected (whenever possible): date of birth, gender, gestational age at birth, multiplicity, birth weight, history of blood component transfusions and their dates.

The male-to-female ratio was 106.8:100. The proportion of premature infants (born before 37 weeks of gestation) was 5.86% (95% CI: 5.74–5.99%) (Table 1 supp l.). The median value for birth weight for the whole group was 3,360 g (min. 450 g, max. 5,500 g). The preterm newborns' percentage as well as the male-to-female ratio were comparable to the data available for the whole Russian Federation.

Age terminology during the perinatal period was used according to the American Academy of Pediatrics recommendations [21].

TREC/KREC Measurement

The NBS tests were performed using Eonis™ SCID-SMA kit (Wallac Oy, Turku, Finland), which is intended for the

semi-quantitative determination of TREC and KREC as well as the qualitative detection of the exon 7 deletion of *SMN1* gene, and *RPP30* (Ribonuclease P/MRP Subunit P30) gene as an internal reference. The 3.2 mm DBS were punched directly into a 96-well plate using a DBS Puncher™ (Perkin Elmer). DNA elution was performed in JANUS Extraction instrument (Perkin Elmer, Turku, Finland), and the real-time PCR analysis was carried out on Applied Biosystems QuantStudio Dx instruments (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's recommendations.

TREC and KREC concentrations (copies/10^5 cells) were calculated by the Eonis™ Analysis software (Wallac Oy, Turku, Finland). The TREC and KREC values of 100 copies per 10^5 cells were chosen as cut-off for the pilot study, based on the manufacturer's recommendations and published data.

Values were considered non-valid when RPP30 Ct < 15.0, Ct > 32.00, or no Ct.

Screening Algorithm

Samples were initially screened by quantifying TREC and KREC in dried blood spots (DBS). Samples with TREC/KREC concentrations above the cutoff value were considered NBS negative. In cases of inconclusive results (*RPP30* Ct < 15.0 or Ct > 32.00), a repeat test was performed in duplicate, and if the results were inconclusive again, a new DBS card was requested. If the TREC and/or KREC result was below the cutoff, samples were subjected to retesting. Retesting involved taking two additional punches from the same Guthrie card. If both punches were below the TREC and/or KREC cutoff, the sample was considered positive.

For preterm newborns with TREC/KREC below cutoff value, a second sample was taken 4 weeks after the first one and if still positive, additionally until 42 weeks of postmenstrual age and subsequent evaluation followed the protocol used for the full-term newborns. Positive screening results necessitated validation which included medical history of the family, clinical investigation of the baby, lymphocytes subsets analysis via flow cytometry (FC) and TREC/KREC retesting in whole blood.

Flow Cytometry

EDTA blood samples were analyzed for lymphocyte cell surface markers according to the lyse-no-wash manufacturer's protocol (Beckman Coulter, US) for multi-color flow cytometry method, using a Beckman Coulter CytoFLEX flow cytometer and a custom dry format DURA Innovations antibody panel (LUCID product line, Beckman Coulter, US). The analyzed lymphocytes subsets included T cells (CD3/CD4/CD8), naïve T cell (CD3/CD45RA/CD197), B cells (CD19), and NK cells (CD16/CD56). Typical SCID was suspected in patients with CD3+ T cell count below 300 cells/μL, leaky SCID was suspected in patients with CD3+ T cell count above 300 cells/μL but naïve T cells subset below 60%. IEI with B lymphocytes defects were suspected in patients with CD19+ lymphocytes fewer than 400 cells/μL. These levels were determined based on the immunophenotyping of confirmed SCID/agammaglobulinemia patients diagnosed prior to the PID newborn screening implementation.

Genetic Analysis

Newborns with suspected/diagnosed IEI based on the results of flow cytometry validation underwent molecular genetic testing. The initial step utilized multiplex ligation-dependent probe amplification (MLPA) analysis for detection of 22q11.2 deletion using SALSA MLPA Probemix P250 DiGeorge (MRC Holland, the Netherlands) according to the manufacturer's recommendations. If the results of the MLPA analysis were normal, whole-exome sequencing (WES) was performed on genomic DNA samples from the patients as described elsewhere [22]. Causative variants discovered by WES were validated by Sanger sequencing in the patient and parents where appropriate. If no causative variants were detected the whole genome sequencing was performed when available.

Metaphase chromosome spreads were prepared from PHA stimulated lymphocyte cultures by standard methods before FISH analysis. A deletion of the chromosome 22q11.2 region is determined by FISH, using the dual-color *TBX1* (22q11) (Spectrum Red) and *SHANK3* (22q13) (Spectrum Green) locus specific probe (Leica Biosystems, Kreatech, US) for the DGS region according to the manufacturer's recommendations.

Retrospective TREC Analysis after Blood Components Transfusion

In order to assess the impact of non-irradiated blood components transfusions on TREC numbers, documentation of the patients with verified SCID, followed in the D. Rogachev National Medical Research Centre for Pediatric Hematology, Oncology and Immunology (Moscow, Russia) prior to the NBS IEI screening introduction (from January 2012 to December 2021) were retrospectively analyzed. No SCID patients with a history of full blood exchange were identified. Fourteen SCID patients who underwent non-irradiated red blood cells (RBC) transfusions prior to TREC/KREC measurements that were performed as part of the institutional routine SCID protocol

laboratory investigation were identified and their data were analyzed. TREC/KREC assay was performed in these patients as described elsewhere [23].

Statistical Analysis

Data was collected and analyzed using GraphPad Prism 8.0.1 (GraphPad Software, San Diego, California USA). Data was presented as median with interquartile range unless contrary provision is made elsewhere. Fisher's 95% confidence interval for proportional data was calculated using WinPepi v. 11.65 software [24].

Results

Among 202,908 newborns most had TREC and KREC values above the cut off level. While TREC numbers varied and increased with gestational age of newborns, there was no such correlation for KREC concentration (Fig. 1). Extremely preterm newborns constituted the most divergent group (Fig. 1). Nevertheless, most of preterm newborns normalized TREC values by the postmenstrual age of 37 weeks (Fig. 2).

1,057 newborns were NBS positive at the first testing (5.21‰ of total), and 134 at the second (0.66‰ of total).

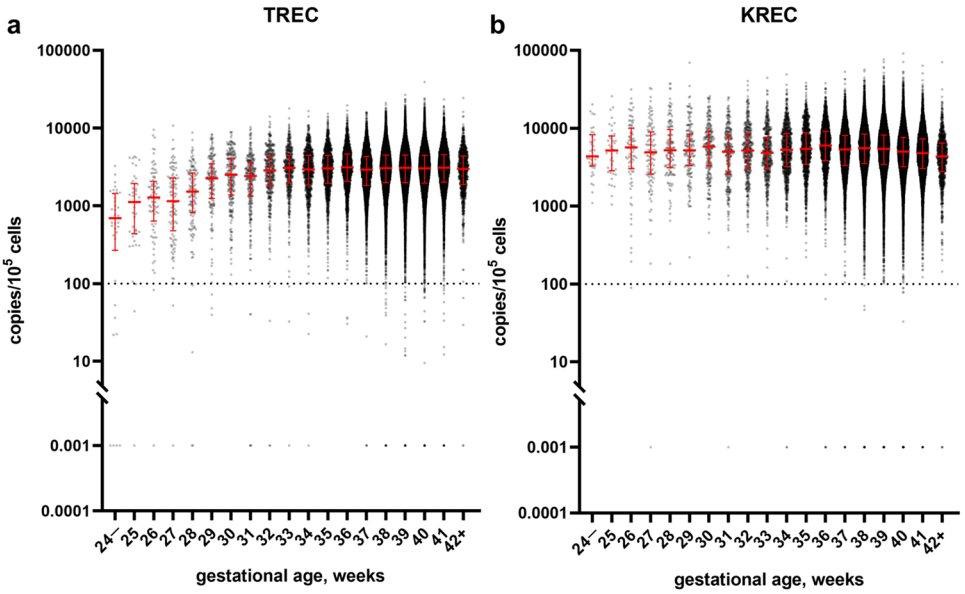

Fig. 1 TREC (**a**) and KREC (**b**) concentrations in newborns of different gestational age. Red lines represent median and interquartile range. Dotted line represents cut-off value of concentrations of analytes. Note $\log_{10}$ scale on the Y axis

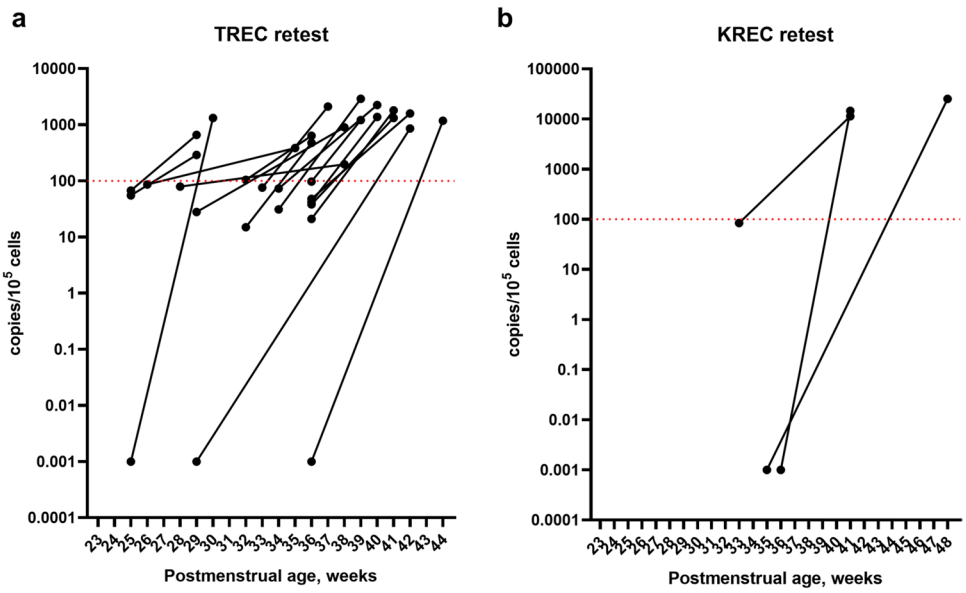

Fig. 2 Dynamics of TREC (**a**) and KREC (**b**) concentrations in preterm newborns with positive NBS who normalized these parameters on the retest. Red lines represent cut-off value of TREC/KREC concentrations. Note $\log_{10}$ scale on the Y axis

Among the 134 newborns who were positive on retest, 55 were premature (0.27‰ of total) and were to be retested within median 26 days from the initial test (IQR = 23–41). 26/55 did not survive to retest or refused further testing (0.13‰ of total), and 14/55 showed a repeated decrease in TREC and/or KREC (0.07‰ of total) on retest. Fifteen premature infants (0.07‰) normalized TREC or KREC values at a median of 38 weeks of postmenstrual age (IQR = 32–41) (Fig. 2).

After retesting of term and preterm infants a total of 93 children were NBS positive (0.46‰), with parameters below the cut-off value as follows: TREC in 77 neonates, TREC and KREC in 7, and KREC in 9 neonates. Nine families refused further testing or were lost to follow up (Fig. 3).

Of the 84 neonates who underwent flow cytometry validation, normal values of lymphocyte subpopulations in peripheral blood were detected in 66 neonates (0.33‰ of total). T and/or B cell lymphopenia was confirmed in 18 newborns (0.09‰ of total). Subsequent genetic testing identified known types of primary or secondary immunodeficiencies in 15 of them: autosomal recessive agammaglobulinemia in one, 22q11.2DS syndrome in 4, SCID in 7, *FOXN1* deficiency in one, Down syndrome in one patient and syndromic T-cell lymphopenia in one (Table 1). The genetic defect was not identified in one patient with T−B+NK+ SCID and syndromic features and in one patient with profound T and B cell lymphopenia and multiple life-limiting congenital malformations. Three more patients with low TREC represented a group of transient lymphopenia. They normalized TREC and lymphocyte counts within the first 2–5 months of life, in two of them no genetic defects have been identified, in one MLPA results were normal and no further genetic testing has been performed (Table 2).

In patients with genetically validated SCID, defects of the following genes have been identified: *IL2RG* ($n = 2$), *AK1* ($n = 1$), *RAG1* ($n = 1$), *RAG2* ($n = 1$), *ADA* ($n = 1$) (Table 1). In all cases but one genetic variants have been previously described and confirmed to be pathogenic. In one patient with T−B−NK+ SCID, WES analysis revealed only one pathogenic single nucleotide variant in exon 2 of the *RAG1* gene: NM_000448.3:c.2210G>A (HGMD: CM981696), resulting in nonsynonymous substitution in the highly conserved position p(Arg737His). In search for the second variant in the gene, whole genome sequencing was performed, which revealed a 287-nucleotide insertion of the Alu repeat of AluYb8 class sequence in the reverse orientation with a single-nucleotide substitution chr5:g.163081823A>G within the Alu-repeat sequence, a 48-nucleotide-long polyA tail, and a dinucleotide duplication of the insertion site (chr11:g.36597625_36597626CT) into exon 2 of the *RAG1* gene within the open reading frame at position NM_000448.3:c.2772_2773ins (Table 1). This insertion can disrupt the open reading frame of the *RAG1* gene to form a nonfunctional protein and is predicted to be likely pathogenic. Segregation analysis confirmed that the variants were located *in trans*. These mobile element insertions (MEI) are known to cause genetic diseases in humans, pathogenic MEI have been shown to be involved in various conditions and are estimated to account for approximately 0.03–0.1% of pathogenic variants responsible for genetic diseases [25].

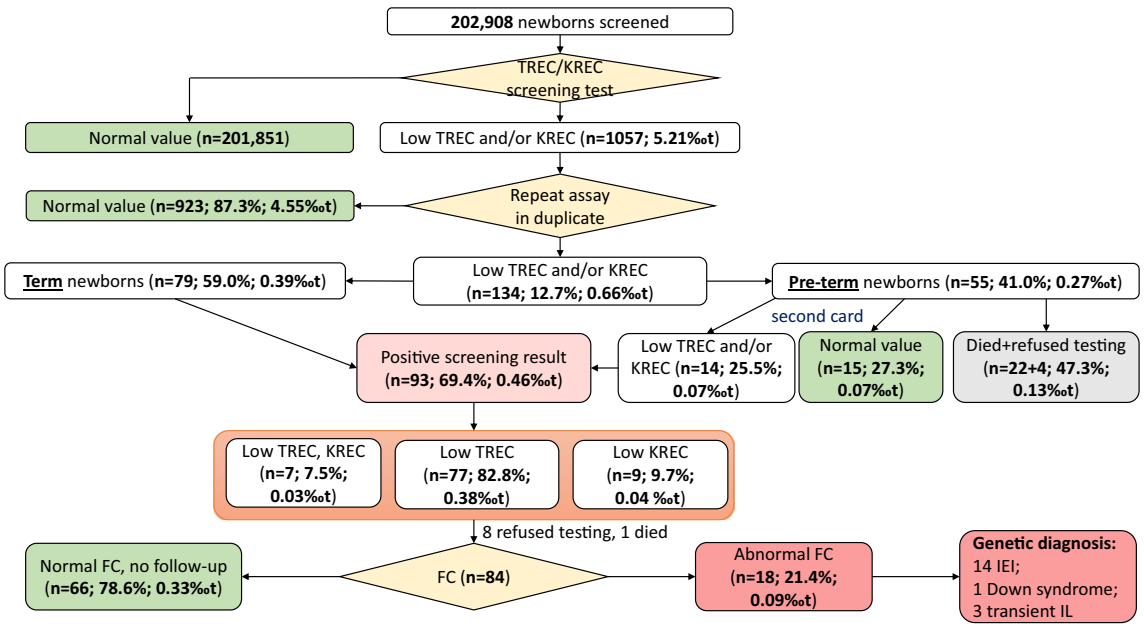

Fig. 3 IEI NBS screening flowchart and statistics. FC – flow cytometry, IL – idiopathic lymphopenia, ‰t – ‰ of total

Table 1 Patients with confirmed immunodeficiencies

ID	sex	TREC/KREC values on initial testing (on retest), copies/10^5 cells	Immunophenotype	Performed genetic tests	Genetic test results (hg19)	HGMD accession number	Clinical significance of revealed genetic variants	Diagnosis (OMIM)
Low TREC, KREC								
16801/2	f	0/0 (0/0)	T−B−NK−	WES, Sanger sequencing	NM_000022.4(*ADA*):c.[872C>T];[872C>T], p.(Ser291Leu), hmz	CM920007	PAT	*ADA*-SCID (#102700)
20745/2	f	0/0 (0/0)	T−B−NK+	WES, WGS, Sanger sequencing	NM_000448.3(*RAG1*):c.[2210G>A];[2772_2773insAlu†], p.[(Arg737His)];[?], cmp htz	CM981696/–	PAT/LPAT	*RAG1*-SCID (#601457)
28886/2	m	0/0 (0/0)	T−B−NK−	WES, Sanger sequencing	NM_000536.4(*RAG2*):c.[685C>T];[685C>T], p.(Arg229Trp), hmz	CM010087	PAT	*RAG2*-SCID (#601457)
734261802	f	253/32 (not performed)	TlowBlowNK+	Karyotyping	47,XX,+21			Down syndrome (#190685)
Low TREC								
10607/2	m	0/5565 (0/25105)	T− B+NK−	MLPA, WES	NM_001625.4(*AK2*):c.[1A>G];[1A>G], p.(Met1Val), hmz	CM090012	PAT	Reticular dysgenesis (#267500)
20899/2	m	79/9462 (139/31139)	TlowB+NK+	MLPA, WES	NM_000206.3(*IL2RG*):c.[982C>T];[0], p.(Arg328Ter), hemi	CM1720475	PAT	TlowB+NK+SCID (#312863)
21412/2	m	0/5542 (0/6964)	T−B+NK−	Sanger sequencing	NM_000206.3(*IL2RG*):c.[222G>C];[0], p.(Trp74Cys), hemi	CM011373	PAT	T−B+NK− SCID, (#300400)
13575/2	f	0/21757 (0/40034)	T−B+NK+	Karyotyping, MLPA, WES, WGS	46,XY	–	–	T−B+NK+SCID, 46,XY sex reversal
15187/2	m	0/4515	TlowB+NK+	MLPA	NC_000022.10:g.(?_19241636)_(21349221_?)del (22q11.2delA–D), htz		PAT	22q11.2DS (#188400)
19730/2	m	38/3973 (112/12397)	TlowB+NK+	MLPA	NC_000022.10:g.(?_19241636)_(21349221_?)del (22q11.2delA–D), htz		PAT	22q11.2DS (#188400)
23790/2	m	0/2915 (0/7143)	TlowB+NK+	MLPA	NC_000022.10:g.(?_19241636)_(21349221_?)del (22q11.2delA–D), htz		PAT	22q11.2DS (#188400)
786/3	m	7/24267 (99/45323)	TlowB+NK+	MLPA	NC_000022.10:g.(?_19241636)_(21349221_?)del (22q11.2delA–D), htz		PAT	22q11.2DS (#188400)
403/3	m	0/4022 (15/1398)	TlowB+NK+	FISH, WES	–	–	–	Syndromic T-cell lymphopenia
16235/2	f	26/6716 (42/9237)	TlowB+NK+	MLPA, WES	NM_003593.3(*FOXN1*):c.[85C>T];[=], p.(Gln29Ter), htz	–	LPAT	T-lymphopenia, infantile, with or without nail dystrophy, AD (#618806)
Low KREC								
24241/2	f	3119/0 (6209/80)	T+B−NK+	WES	NM_020070.4(*IGLL1*):c.[425C>T];[425C>T], p.(Pro142Leu), hmz	CM2020943	PAT	Autosomal recessive agammaglobulinemia type 2 (#613500)

hmz – homozygous; htz – heterozygous; cmp htz – compound heterozygous; hemi – hemizygous; PAT – pathogenic, LPAT – likely pathogenic, VUS – variant of unknown clinical significance; † – allele with Alu-repeat insertion chr11:g.36597626_36597627ins[[chr5:g.163081634_163081920{163081823A>G};A[48]]inv;36597625_36597626] resulting in NM_000448.3:c.2772_2773ins[[chr5:g.163081634_163081920{163081823A>G};A[48]]inv;2771_2772]

However, this is likely to be a substantial underestimation, since it is challenging to detect this type of variant using routine molecular tests [26, 27].

Another interesting case revealed by NBS was a phenotypically female patient with T−B+NK+ SCID who demonstrated to have normal male karyotype, 46,XY, thus diagnosed additionally with 46,XY-sex reversal syndrome (SRS). Whole exome and whole genome sequencing failed to identify causative defects for SCID and/or SRS in this patient.

Table 2 Patients with transient idiopathic lymphopenia

ID	Sex	TREC/KREC values on initial testing (on retest), copies/10^5 cells	Immunophenotype	Performed genetic tests	Genetic test results (hg19)	HGMD accession number	Clinical significance of revealed genetic variants	Diagnosis (OMIM)
Low TREC								
17042/2	m	33/3275 (14/6795)	TlowB+NK+	MLPA, WES	–	–	–	Transient lymphopenia
17877/2	m	72/8770 (132/121)	TlowBlowNK+	MLPA	–	–	–	Transient lymphopenia
21474/2	f	30/946 (108/260)	TlowBlowNK+	MLPA	–	–	–	Transient lymphopenia

To validate the NBS protocol with regards to newborns who underwent blood transfusion prior to testing, we performed retrospective analysis of 14 SCID patients (T−B− —4/14; T−B+ —10/14) ages 1–8 months (Me—5) identified before IEI NBS introduction in whom TREC/KREC testing was performed as part of their diagnostic work up. Patients received 1–3 (Me—1) RBC transfusions prior to TREC/KREC testing. The interval between the first transfusion and testing was 2–49 days (Me—7). None of the patients had clinical signs of transfusion-associated graft versus host disease at the time of the assay. In the whole group the median TREC level was 0 copies/10^5 leucocytes (0–23). In four SCID patients with T−B− SCID the median KREC level was 0 copies/10^5 leucocytes (0–46).

Discussion

In this pilot study, the newborn screening for IEI was performed in 8 regions of Russian Federation for 202,908 newborns. This number accounted for 96.1% of newborns in these regions, and about 1/6 of all newborns in Russia during the study period.

Based on the bulk of the previously published data [28, 29] in the current study the cut-offs for both TREC and KREC were determined to be 100 copies/10^5 cells. As the resulting statistics of T cell deficiencies that were detected during the pilot NBS corresponded to the previously published results, we concluded that these cut-offs seem to be effective for the target group of IEI. It is worth mentioning that all SCID or XLA identified through NBS have demonstrated near zero values of the respective analytes, hence substantially lower than cut-off values (Table 1).

Using these cut-off values we have obtained 5.21‰ of samples requiring repeated measurements. This retest rate is higher than previously reported 0.00–4.10‰ [29]. We have found some systematic problems at the pre-analytical stage with DBS cards on a regional basis. Repeated measurement decreased the referral rate to 0.66‰, with 87.30% of the initially abnormal results being normal after retesting. These referral rate is in range with other pilot studies [29].

An overall distribution of TREC values demonstrated difference in TREC concentrations depending on gestational age which is in concordance with the literature data [30, 31]. KREC values showed less variability with respect to the gestational age. This could be indicative of earlier maturation of B-lymphocytes during ontogenesis [32].

The birth prevalence of T and B cell immunodeficiencies that were detected via NBS in the current study was 1:14,493 newborns (95% CI: 1:8,621–26,316). Moreover, the birth prevalence of the most severe IEI form, severe combined immunodeficiency, was 1:28,986 newborns (95% CI: 1:14,084–71,428). It should be noted that these figures are higher than those obtained in other screening programs, which could possibly be due to a relatively small cohort of newborns screened [33, 34]. Interestingly, majority of the patients with autosomal recessive SCID were homozygous for the respective genetic variants. Though Russia does not have a high rate of documented consanguinity, implementing IEI NBS in larger cohorts of newborns in the future might demonstrate presence of hot-spot variants of SCID genes in Russian population as has been previously demonstrated for *RAG1* gene in Slavs [35].

One of the questions raised during the NBS protocol set up concerned validity of the TREC/KREC values after blood products transfusion. There are currently no validated recommendations for TREC or KREC testing after blood transfusions, yet many studies exclude those cases from the subsequent analysis [10]. The issue is especially relevant for the exchange transfusions in which all newborn blood cells including lymphocytes are substituted for that of an adult donor [36]. It is known that TREC and to lesser extent KREC values decrease substantially with age [37]. Whole blood exchange transfusions are fairly rare [38] hence our study was not able to study TREC/KREC values after the blood exchange and a systematic study addressing this issue

is required. Another concern is transfusion of non-irradiated packed RBC, still used in some countries. Theoretically it can lead to higher TREC/KREC values in SCID patients in the cases of engraftment of the donor lymphocytes contained in the red blood cells preparations [39]. Based on our retrospective analysis of SCID patients we conclude that transfusion of non-irradiated RBC does not interfere with the validity of the IEI NBS screening and should not be postponed.

The categories of newborns detected by IEI NBS screening were not different from the previously published [40]. Patients with low KREC are of special interest as the controversy about cost-effectiveness of their use in NBS exists. KREC below cut off were demonstrated in 8 newborns with normal B-cell counts, as well as in a patient with agammaglobulinemia (AGG) and absent B cells. Though majority of patients with AGG are males with *BTK* gene defects [41], the only patient found by NBS was a female with a homozygous *IGLL1* gene variant previously described in patients with AR agammaglobulinemia [42]. Homozygous state of the variant identified in the patient born to non-consanguineous parents again could point to a common variant in Russian population. Further studies are required to test this hypothesis.

In conclusion, the pilot study of IEI NBS screening demonstrated the feasibility of the implemented protocol, including combined TREC/KREC testing, repeat measurements, and inclusion of all newborns, including the ones who received unirradiated blood components, in the NBS. We demonstrate that TREC/KREC assay is informative in SCID patients who received transfusions of non-irradiated RBC. Therefore, these newborns should not be delayed for NBS. Our study revealed certain intriguing genetic tendencies that require verification in larger cohorts of newborns.

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Author Contributions A.V.M., R.A.Z., S.V.V., A.S., S.I.K. designed most of the studies. A.V.M., I.Yu.E., N.V.B. performed screening assays. A.A.M., Y.R., D.P. carried out much of the immunological work. A.V.M., O.P.R., A.A.O., V.V.Z., T.BC., T.S.B., O.A.S., A.V.P., Z.G.M., M.E.M., N.V.S. performed DNA diagnosis. S.S.L., M.B.K., E.S.D., E.V.K. participated in retrospective TREC analysis after blood components transfusion. D.A.M., D.H.S., S.A.M., E.Yu.B., G.I.Y., I.S.T., Y.V.G., N.A.I., L.R.N., E.V.S., T.I.B., O.S.R. performed screening in the regions. A.V.M., I.Yu.E., A.A.M. analyzed the data. A.V.M., I.Yu.E., A.A.M., A.S. drafted the manuscript, and all authors approved the final manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

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