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Beyond TREC: Pivotal role of tandem TREC/KREC assay in Czech SCID NBS pilot programme

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Abstract

Background: Severe combined immunodeficiency (SCID) is a fatal but treatable inborn error of immunity (IEI). Newborn screening (NBS) using T-cell receptor excision circles (TREC) has been adopted globally, with very few countries incorporating kappa recombination excision circles (KREC) to also detect early B-cell development disorders, such as X-linked agammaglobulinemia (XLA).

Objective: To evaluate the effectiveness of a 2-year pilot SCID NBS program in the Czech Republic, emphasising the utility of combined TREC/KREC screening.

Methods: Between January 2022 and December 2023, a dual TREC/KREC NBS pilot was conducted across the Czech Republic, alongside spinal muscular atrophy (SMA) screening. Approximately 200,000 newborns were screened using quantitative real-time PCR on dried blood spots collected 48–72 h after birth.

Abbreviations: CC, clinical centre; CD, cluster of differentiation; CMV, cytomegalovirus; DBS, dry blood spot; HSCT, haematopoietic stem cell transplantation; IEI, inborn error of immunity; IgRT, immunoglobulin replacement therapy; KREC, kappa recombination excision circle; NBS, newborn screening; NK, natural killer; SCID, severe combined immunodeficiency; SMA, spinal muscular atrophy; TEMRA, terminally differentiated effector memory T cells; TPMT, thiopurine S-methyltransferase; TREC, T-cell receptor excision circle; XLA, X-linked agammaglobulinemia.

Results: The pilot referred 58 newborns, identifying 21 cases of IEI, including two SCID cases, with an overall incidence of TREC/KREC screenable IEI of 10.5/100,000 newborns. SCID incidence was 1/100,000. KREC screening proved invaluable, detecting 10 cases of congenital agammaglobulinemia including novel non-XLA forms, which increased the estimated incidence of agammaglobulinemia in the Czech Republic six-fold. Over one-third of low KREC results were linked to maternal immunosuppression. **Conclusion:** The Czech pilot demonstrated the effectiveness of integrated TREC/KREC NBS in detecting both T- and B-cell immunodeficiencies. As of 2024, SCID and SMA screening are included in the nationwide NBS, with KREC screening significantly improving early detection of B-cell disorders.

KEYWORDS
agammaglobulinemia, dried blood spot, IGLL1, KREC, SCID, screening, severe combined immunodeficiency, TREC, XLA

1 | INTRODUCTION

Newborn screening (NBS) for SCID (severe combined immunodeficiency), a lethal yet curable inborn error of immunity (IEI), by TREC (T cell receptor excision circles) detection was initiated in 2008 in Wisconsin, USA.[1] Since then, the TREC-based SCID NBS has become an umbrella term for screening severe errors of T cell development, encompassing not only SCID but also other non-SCID diagnoses. At the time of this manuscript writing, over 35 countries worldwide included SCID screening in their nation-wide NBS programmes, on a regional basis or are in the process of pilot testing its implementation. In parallel, a handful of countries simultaneously instituted KREC (kappa recombination excision circles) assays to screen for inborn errors of early B cell development, such as X-linked agammaglobulinemia (XLA) or other forms of agammaglobulinemia (implemented in Sweden, Spain, Poland, Germany, Ukraine, Switzerland, Slovakia and Russia[2]). The overall global experience univocally substantiates the use of TREC screening as a robust tool with high sensitivity for SCID and specificity for SCID/CID ranging between 13% and 65%, depending on the country, as improved outcomes of NBS-diagnosed SCID patients are being reported.[2–13]

Here, we present the results of a two-year (January 2022–December 2023) population-based SCID screening pilot programme in the Czech Republic. Within the country's population of approximately 10.9 million, approximately 100,000 children are born on average each year.[14] The incidence of SCID prior to NBS was estimated at 1–2/100000 newborns (1–2 patients/year) and the incidence of XLA was 1:120000. The SCID NBS was carried out using dual TREC/KREC evaluation, alongside the screening pilot programme for spinal muscular atrophy (SMA), taking advantage of both tests utilising DNA analysis as a primary screening method. Based on the successful pilot phase, as of January 1, 2024, SCID and SMA NBS were integrated into the population-wide NBS in the Czech Republic, which now spans a total of 20 severe inborn diseases.

Key message

The Czech pilot demonstrated excellent effectiveness of integrated TREC/KREC NBS in detecting both T- and B-cell immunodeficiencies. As of 2024, SCID screening is included in the nationwide NBS, with KREC screening significantly improving early detection of B-cell disorders.

2 | METHODS

The pilot study of SCID NBS in the Czech Republic was designed by the Committee for SMA and SCID screening established by the Czech Ministry of Health as opt-in, with voluntary participation offered to all mothers giving birth from January 1st, 2022, to December 31st, 2023, in all neonatal wards in the Czech Republic. All mothers were informed about the ongoing pilot project, received spoken and written information, and were offered—at no additional intervention to the child—to include SCID and SMA in their child's neonatal screening. Upon the signature of the informed consent, dry blood spot (DBS) cards were collected at 48–72 h of age, regardless of gestational age/birth weight.

Samples were processed using quantitative real-time polymerase chain reaction (qPCR) at two centres, in Prague and Brno, testing samples from Bohemia and Moravia respectively. TRECs and KRECs were tested using a commercial kit (PerkinElmer, Waltham, Mass), with copies quantified per 100,000 cells using the reference gene *RPP30* for every sample as a quality control. Samples with TREC or KREC <10 copies/100,000 cells were referred directly to clinical centres at the Department of Immunology, University Hospital in Motol, Prague and the Department of Pediatrics, Faculty Hospital Brno, Brno. Samples with TREC or KREC 10–100 copies/100,000 cells were retested from a second DBS card, obtained from the child's general practitioner or other current healthcare provider

within the first 2 weeks of life—prior to administration of live vaccines, and only referred to clinical centres if the repeated measure fell below the 100 TREC or KREC copies/100,000 cells threshold.

In preterm infants with birth weight <1500 g and first result of TREC or KREC <10 copies/100000 cells, a second DBS card was collected at 2 weeks or when >1500 g of weight, whichever came first, and referred to clinical centre if TREC or KREC remained <100 copies/100000 cells. If the child remained <1500 g at 2 weeks of age, a third DBS card was collected when this target weight was reached.

Further approach of the reference centre was tailored based on the type of the result, as well as a brief clinical history obtained from primary caregivers or family physicians via a phone call. In case of low TRECs in a full-term infant, the first visit was scheduled as soon as possible, usually within days from the referral. In case of low KRECs but normal TRECs, the first visit was scheduled for approximately 1 month of the patient's age, due to the lower risk of clinical complications of B cell lymphopenia in the setting of maternally transferred antibodies (Table 1).

At the first visit, all patients were examined, complete past medical, pregnancy-related and family history was taken, with particular focus on sibling and maternal-related morbidity. Laboratory tests included complete blood count with white blood cell differential, basic lymphocyte subtyping (T/B/NK cell enumeration), T lymphocyte (recent thymic emigrant, naïve, central, effector and TEMRA T cells, gamma delta T cells, alpha beta double negative T cells and activated DR+ T cells) and B lymphocyte (naïve, transitional, switched memory, marginal zone-like, plasmablast and CD21low) phenotype assessment using flow cytometry (T cell analysis was performed using a custom-made dry reagent tube (EXBIO, Czechia), B cell analysis was performed using the DURAClone IM B Cell dry reagent tube (Beckman Coulter Life Sciences, USA)), serum immunoglobulins and maternal cytomegalovirus (CMV) status. Blood samples for DNA isolation and sequencing were also obtained from patients and both parents upon first visit, to facilitate faster processing in case of pathological first-tier cytometry results.

Genetic assessments were performed at the Centre for Cardiovascular Surgery and Transplantation, Brno and the Department of Biology and Medical Genetics, 2nd Medical Faculty of Charles University and University Hospital Motol, Prague. The test selection algorithm was guided by the TREC/KREC result and the phenotype of the patient (Figure S1).

3 | RESULTS

3.1 | NBS increases the incidence of TREC/KREC screenable IEI in the Czech Republic up to 10/100,000; SCID incidence remains unchanged, and XLA incidence increases dramatically

Between January 1st, 2022, and December 31st, 2023, a total of 198,675 samples were tested in the Czech Republic as a part of the initial feasibility pilot study, according to the methodology outlined above. The opt-in rate was >92%. The number of neonates identified through each referral pathway is illustrated in Figure 1.

Ultimately, this approach resulted in 58 neonates being referred to clinical centres, constituting a referral rate of approximately 0.029%. Of the 58 referrals, 21 had low TREC and normal KREC, 33 had low KREC and normal TREC, and 4 had low both TREC and KREC (Table 2). Amongst them, 21 IEI were identified, indicating an overall incidence of TREC/KREC screenable IEI in the Czech Republic of 10.5/100000 (for the list of identified monogenic variants, see Table S1). The most common causes of abnormal NBS in the non-IEI group were prematurity, perinatal infections and intrauterine exposure to maternal medication. Seven children identified with NBS died before referral to the clinical centers, all of whom died due to direct sequelae of extreme prematurity.

Amongst the IEI, two patients with SCID (CD3ε deficiency and maternal diabetes-induced complete DiGeorge syndrome with no underlying genetic defect) were identified, forming the core outcome cohort of the NBS and ascertaining the SCID incidence to be approximately 1:100000, which remained unchanged with the screening.

Furthermore, seven patients were diagnosed with 22q11.2 deletion syndrome, with mild (>400/μL CD4 T cells and >200/μL CD8 T cells, 3/7 patients) and significant (<400/μL CD4 T cells or <200/μL CD8 T cells, 4/7 patients) T cell lymphopenia.[15] Two patients with thymic hypoplasia due to *FOXN1* haploinsufficiency and *TBX1* deficiency were found, both presenting with significant T lymphopenia, but with preserved naive T cell compartment and not fulfilling diagnostic criteria for SCID (e.g. athymia, hair and nail dysplasia in *FOXN1* SCID[16–18] and athymia, craniofacial dysmorphia, sensorineural deafness, hypocalcaemia in *TBX1* deficiency[18–20]). Both patients remain on conservative prophylactic antibiotic treatment only, pending improvement of cellular immunity. One patient was found to harbour a complex chromosome 9 aberration, which has not been described in association with IEI, and neither the patient's clinical course nor immunological work-up indicates immunodeficiency. In this case, we hypothesize that the low TRECs were a result of perinatal sepsis, such as in other patients with perinatal infections and low TRECs but no overt immunodeficiency.

Ten patients with agammaglobulinemia were identified in the low KREC cohort, out of whom five had X-linked agammaglobulinemia due to a *BTK* pathogenic variant, four had autosomal recessive (AR) agammaglobulinemia caused by *IGLL1* pathogenic variants, and one had autosomal dominant agammaglobulinemia (AD) caused by a *TCF3* pathogenic variant.

In both the low TREC and low KREC group, some patients presented with decreased T cells (1×) or decreased B cells (5×) less severe than SCID/agammaglobulinemia, yet neither a conclusive genetic diagnosis nor any confounding factor could be established. Those patients are listed as idiopathic T or B cell lymphopenia and remain under close monitoring, receiving prophylactic antimicrobial treatment depending on infectious morbidity and T-cell/immunoglobulin levels.

Finally, in two children, the peripheral blood cytometry did not verify the low KREC finding, as B cells were normal, and as such,

TABLE 1 Interpretation of TREC/KREC values and consequent actions within the Czech SCID NBS program.

TREC evaluation				
TREC copies number per 100,000 cells	**Birth weight <1500 g**	**Interpretation**	**Action by screening laboratory**	**Action by clinical centre**
>100	No	Normal	None	None
<10	No	Urgent positive	Urgent referral to CC	Immediate clinical visit, fresh blood sampling for immune phenotyping (within days).
<10	Yes	Positive	Direct referral to CC	No immediate action taken, wait for 2nd DBS in 2 weeks or when >1500 g.
10–100	No	Positive	Immediate 2nd DBS request, then referral to CC if TREC still <100, otherwise no action taken.[a]	Scheduling of clinical visit with fresh blood sampling for immune phenotyping before 1 month of age.
10–100	Yes	Positive	2nd DBS in 2 weeks or when >1500 g, referral to CC if TREC still <100, otherwise no action taken.	Depending on current weight: • IF >1500 g: Scheduling of clinical visit, fresh blood sampling for immune phenotyping usually within 2 weeks. • IF <1500 g: No immediate action taken, wait for 3rd DBS when >1500 g.
KREC evaluation				
KREC copies number per 100,000 cells AND normal TREC	**Birth weight <1500 g**	**Interpretation**	**Action by screening laboratory**	**Action by CC**
>100	No	Normal	None	None
<10	No	Positive	Direct referral to CC	History of maternal immunosuppressive medication in pregnancy • IF NO: Scheduling of clinical visit, fresh blood sampling for immune phenotyping usually within 2 weeks. • IF YES: Scheduling of clinical visit, fresh blood sampling for immune phenotyping at 1 month of age.
<10	Yes	Positive	Direct referral to CC	No immediate action taken, wait for 2nd DBS in 2 weeks or when >1500 g.
10–100	No	Positive	Immediate 2nd DBS request, then referral to CC if KREC still <100, otherwise no action taken.[a]	History of maternal immunosuppressive medication in pregnancy • IF NO: Scheduling of clinical visit, fresh blood sampling for immune phenotyping usually within 2–3 weeks. • IF YES: Scheduling of clinical visit, fresh blood sampling for immune phenotyping at 1 month of age.
10–100	Yes	Positive	2nd DBS in 2 weeks or when >1500 g, referral to CC if KREC still <100, otherwise no action taken.	Depending on current weight: • IF >1500 g: Scheduling of clinical visit, fresh blood sampling for immune phenotyping usually within 2 weeks. • IF <1500 g: No immediate action taken, wait for 3rd DBS when >1500 g.

Abbreviation: CC, clinical centre.

[a]Direct referral to CC if general practitioner/primary caregiver is out of reach.

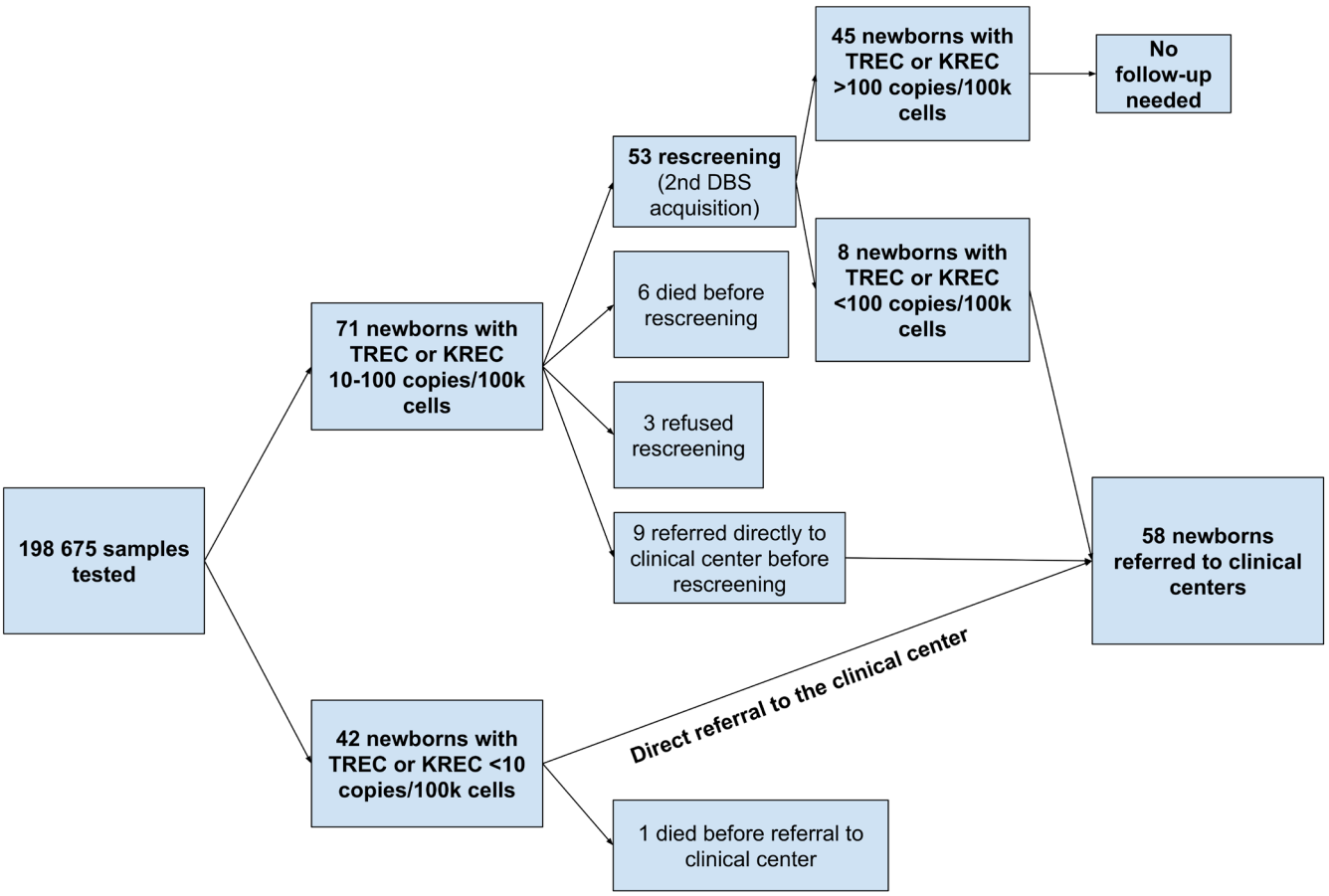

FIGURE 1 Numbers of tested samples and identified/referred neonates.

these samples represent the only "true" laboratory false positive tests in our cohort.

3.2 | NBS improves the age at diagnosis of SCID and the age at haematopoietic stem cell transplantation (HSCT)

3.2.1 | Non-syndromic SCID

In the cohort of 27 SCID patients transplanted in Czechia prior to NBS (between 1995 and 2022), the median time at diagnosis and median time at transplant was 5 months (range: prenatal-52.0 months) and 7 months (range: 2.1–58.0 months), respectively. In comparison, the patient with *CD3ε* deficiency, who was identified through neonatal screening, underwent matched related donor HSCT at 7 weeks of age, prior to any infectious complication.[21]

3.2.2 | Syndromic SCID with athymia

Patients with associated syndromic features are usually diagnosed early even in the absence of NBS, likely due to the striking features of congenital heart disease, craniofacial dysmorphia, hypocalcaemic seizures or other associated pathologies. Prior to SCID NBS, two recorded Czech patients with complete athymia were diagnosed at 2[22] and 1 month[23] of age, respectively, and were treated with partially matched lymphocyte infusions at 6 months of age and thymic transplant at 4 months of age. A patient diagnosed via NBS with maternal diabetes-induced complete DiGeorge syndrome received the final diagnosis at 4 weeks of age and underwent thymic transplant at 3 months of age.

3.3 | KREC screening supports early recognition and treatment of antibody deficiencies

The inclusion of KREC in the screening protocol enabled the early recognition of an unprecedented number of B cell disorders, expanding the scope of utility of the screening programme at no additional cost of biomaterial. In all agammaglobulinemic patients, immunoglobulin replacement therapy (IgRT) has been initiated when IgG <2.5 g/L and prior to infectious complications. Consequently, all remain free of significant infections to date. Drawing on the data that support a competent induction of cellular post-vaccination memory in agammaglobulinemic patients[24–26] and on the scarce existing recommendations,[27–29] all children were recommended to undergo

TABLE 2 The final diagnoses of 58 neonates referred to clinical centers during the Czech SCID NBS pilot programme shown in association with the TREC/KREC status.

Low TREC, normal KREC	*N* = 21
SCID (*CD3E*)	*N* = 1
SCID (complete DiGeorge syndrome)	*N* = 1
22q11.2 deletion syndrome	*N* = 6
Thymic hypoplasia (*TBX1*)	*N* = 1
Thymic hypoplasia (*FOXN1* heterozygous)	*N* = 1
Idiopathic T lymphopenia[a]	*N* = 1
Complex chromosomal aberration	*N* = 1
Prematurity	*N* = 6
Perinatal infection	*N* = 3
Low KREC, normal TREC	*N* = 33
X-linked agammaglobulinemia (*BTK*)	*N* = 5
Autosomal recessive agammaglobulinemia (*IGLL1*)	*N* = 4
Autosomal dominant agammaglobulinemia (*TCF3*)	*N* = 1
Idiopathic B lymphopenia[a]	*N* = 5
Intrauterine exposure to maternal medication	*N* = 12
Prematurity	*N* = 3
Perinatal infection	*N* = 1
Unknown cause of positive screening, normal flow cytometry	*N* = 2
Low TREC, Low KREC	*N* = 4
22q11.2 deletion syndrome/partial DiGeorge syndrome	*N* = 1
Intrauterine exposure to maternal medication	*N* = 1
Perinatal infection	*N* = 1
Prematurity	*N* = 1

[a]At the time of manuscript preparation.

regular vaccination according to the Czech national schedule, with the exception of live vaccines, which were withheld. Additional optional vaccines against *Neisseria meningitidis* and *Streptococcus pneumoniae* were recommended.

3.3.1 | XLA improved recognition

A major improvement in diagnostic delay/recognition of XLA was achieved with the NBS. Based on the data from the Czech National Registry of Primary Immunodeficiencies, mean age at diagnosis of XLA patients prior to the NBS pilot (data from 2002 to 2021) and during NBS was 27 years and 2 months, respectively.

According to the national registry, the incidence of XLA prior to the NBS pilot had been estimated to be 1:120000, with a total of 18 patients diagnosed between 2001 and 2021. Remarkably, within the 2 years of the screening pilot, five patients with XLA were identified, increasing the incidence to 1:38000. Barring a statistically unlikely increase in incidence, this suggests that before the implementation of the NBS, a significant proportion of Czech XLA patients had remained undiagnosed.

3.3.2 | Novel non-XLA agammaglobulinemia: TCF3 and IGLL1 deficiency

Moreover, within the first year of KREC screening, disorders not previously diagnosed in the Czech Republic were identified. Aside from XLA, the ultra-rare TCF3 deficiency was identified, as were four patients with the rare IGLL1 agammaglobulinemia.[30]

3.3.3 | High rate of positive KREC due to secondary causes

Over one third of low perinatal KREC values was caused by exposure to maternal immunosuppression *in uteró* (13 infants). Namely, azathioprine treatment for inflammatory bowel disease was the suspected cause in 12 patients and anti-epileptic medication (lamotrigine and levetiracetam) in 1 patient. When examined at 1 month of age, all of these patients had detectable B cells in peripheral circulation, with supra-physiological proportions of early stages (naïve and transitional B cell subsets), suggesting a swift repopulation of the B cell compartment after the cessation of transplacental exposure to the medication. During the pilot programme, these children were recalled to clinical centres, sampled and followed up; however, given the eventual reconstitution of their lymphocyte counts, the algorithm for this situation is set to be modified. The infants received standard vaccinations, and some were tested for their serologic response following the 2nd dose of tetanus toxoid, with 10/11 tested mounting a sufficient protective specific antibody response. There was no association between KREC levels, maternal dose of azathioprine or maternal thiopurine S-methyltransferase (TPMT) activity.

4 | DISCUSSION AND FUTURE CHALLENGES

The successful implementation of the pilot SCID NBS programme in the Czech Republic marks a significant advancement in early detection and intervention for SCID, severe T cell lymphopenia and agammaglobulinemia patients, with the additional ability to detect some forms of congenital leukemia.[31] Adding to the experience from other countries, this initiative supports the feasibility and effectiveness of integrating dual TREC/KREC screening into routine neonatal care and highlights the added value of incorporating KREC to IEI NBS. However, given a relatively short data collection period (2 years), the presented results must be interpreted with care, particularly those concerning demographic estimates. Importantly, not all IEI are screenable by TREC/KREC assays; therefore, an overall population incidence of IEI may not be extrapolated from TREC/KREC based NBS programmes.

During the first 2 years of NBS, several important insights were gained. Firstly, the referral rate in the Czech programme was 0.029%, which is in line with the recent results in other neonatal

SCID screening programmes.[10,11,13] At the same time, alongside favourable specificity, the test sensitivity was high—as far as the authors are aware, no patient was diagnosed with/suspected of having SCID or severe congenital T cell lymphopenia outside the NBS during the pilot.

Secondly, at least during the first 2 years of NBS implementation, the previously estimated national SCID incidence remained practically unchanged (1:100000), unlike in other countries, which saw a rise of SCID cases, such as the USA, Germany or China.[6,32,33] 4% of all low TREC infants had idiopathic T cell lymphopenia which is lower than, for example, in Germany or California.[2,6] Interestingly, although the estimated prevalence of 22q11.2 microdeletion is 1:3000–6000 live births,[34] which should equal approximately 30–60 newborns with 22q11.2 microdeletion during the 2-year study period, only 7 patients with 22q11.2 deletion were identified, accounting for approximately 10%–20% rate of diagnosis. This aptly reflects the variable immunological phenotype of the 22q11.2 microdeletion syndromes. Importantly, the patients with T cell deficiency less profound than SCID, including 22q11.2 deletion syndromes, *TBX1* and heterozygous *FOXN1* deficiency, presented with absent TRECs and were thus at the screening stage indistinguishable from patients with SCID. Nonetheless, upon clinical visit, these patients only had mildly to moderately decreased T cell counts and in some cases decreased naive and recent thymic emigrant T cells, highlighting the variable severity of these monogenic disorders.[15,16,19,20] The TREC levels did not correlate with T cell counts; however, no patient identified through NBS with low TRECs had normal T cell compartment, with variably decreased mitogen response, proportion of naive and total T cell counts. Thus, clinical and flow cytometric evaluations, not just TREC values + genetic diagnostics, are indispensable for proper risk-stratification of T lymphopenic patients.

Thirdly, the number of congenital B cell deficiencies was dramatically higher than expected and low KREC constituted the majority of the clinical recalls. Although almost 55% of low KREC were "false positives" for IEI, the vast majority of these cases could be readily explained by *in uteró* exposure to maternal medication or prematurity and were all confirmed to be of transient nature. These infants were managed with adjusted urgency and appropriate narrative to avoid unnecessary stress for the patients and their families. Even though B cell deficiencies are less urgently life-threatening compared to SCID (and some have been shown to be markedly variable in severity[30]), delayed diagnosis increases the risk of morbidity due to severe bacterial or viral infections[35] as well as the implicit structural lung damage, thus strongly advocating for the NBS implementation. The cost-effectiveness and efficacy of this inclusion have been internationally debated.[4,36,37] Irrefutably, the unprecedented increase in incidence seen during NBS in the Czech Republic, a country with a high standard of immunologic diagnostics, makes for a compelling argument for incorporating KREC into the NBS, given the unpleasant possible explanation of underdiagnosis and early death before the implementation of the NBS. The current standard of care for XLA patients, that is, life-long immunoglobulin replacement therapy (IgRT), decreases overall morbidity and mortality but fails to prevent all infections, autoimmune complications, and does not prevent the development of chronic lung disease.[38,39] In this context, early diagnosis of agammaglobulinemia through NBS may open the door to other restorative treatments, such as HSCT with dedicated protocols, and, in the future, gene therapy.[40] Moreover, for patients in countries where long-term IgRT may not be available, HSCT, especially when performed early and from a matched-sibling donor or matched-unrelated donor, may be a more cost-effective option per quality-adjusted life year.[41–43]

Fourthly, the earlier diagnosis of SCID in our cohort preceded infectious morbidity, similarly to previous reports from other countries.[44–46] However, there is a lack of consensus on proper antimicrobial prophylaxis, particularly in neonates. While all groups recommend cotrimoxazole-trimethoprim, the recommendations regarding antifungals (such as fluconazole) and antivirals (such as acyclovir) are more diversified.[47–49] Given the non-negligible toxicity of these medications in this age group, considerations need to be given to which medication should be started and when, and international expert consensus should be developed for optimal patient care. Whether patients referred to HSCT through NBS experience improved long-term outcomes remains to be confirmed; however, available data already indicate that higher overall and event-free survival may be expected.[50–52]

Finally, the journey from diagnosis to intervention is fraught with emotional turmoil, anxiety and uncertainty; therefore, the importance of mental health support in the context of SCID NBS cannot be overstated.[53,54] Given the overwhelming complexity of the stress parents/guardians face, we strongly believe that psychological support should be readily offered as part of the SCID NBS program. It is also imperative that physicians involved in NBS are adequately trained in communicating serious diagnoses to families and in dealing with situations of prolonged uncertainty, for example, in cases of idiopathic lymphopenia or during the search for suitable HSC donors. Pertaining to this topic, Table 3 illustrates a case of the first NBS-diagnosed SCID patient narrated from the healthcare providers' vs. the parents´ points of view.

5 | SUMMARY

In the Czech Republic, a 2-year pilot programme for SCID NBS in 2022–2023 employed dual TREC/KREC evaluation to screen for SCID, severe T-cell lymphopenia and congenital B-cell deficiencies. An acceptable referral rate without compromising the sensitivity or excessive false positive samples was demonstrated, along with an unchanged incidence of SCID at 1:100000, but a considerable increase in incidence of congenital B-cell deficiencies (XLA from 1:120000 to 1:38000). Based on our experience, we suggest that the addition of KREC screening is considered in countries that already use TREC screening or plan to start the NBS for IEI.

Overall, the successful implementation of SCID NBS in the Czech Republic demonstrates the benefits of incorporating dual TREC/KREC screening into routine neonatal care. This approach enhances

TABLE 3 The first NBS-diagnosed SCID patient in the Czech Republic—healthcare providers' versus mother's narrative.

Pregnancy and birth	
A healthy term boy AB is born to a mother who contracted COVID-19 and took methyldopa for gestational hypertension during pregnancy. His mother has a rare congenital methemoglobinemia, his father is healthy, his 8-year-old sister has congenital methemoglobinemia and his 10-year-old brother is healthy.	
	
Recall from NBS and the diagnosis	
On the 19th day of life, an abnormal SCID screening result is reported to the Department of Immunology in Motol University Hospital in Prague, with 0 copies of TREC and normal KREC. The family receives a call from the physicians informing the parents of a need for urgent blood testing. The next day, the immune phenotyping from the blood sample confirms profound T cell lymphopenia with lack of naïve CD45RA+ T cells, while B and NK cells are present (i.e. T-B + NK+ immunophenotype). Within the next 3 weeks, panel gene sequencing identifies a homozygous mutation in *CD3E* confirming the diagnosis SCID. Both parents are carriers of one variant, while the brother carries two healthy alleles. Simultaneously, the diagnosis of methemoglobinemia is confirmed in the patient, having been previously suggested by central cyanosis and mild peripheral oxygen desaturation during crying.	During the very first phone call from our pediatrician, saying that a new sample needed to be taken, we were initially in shock. However, we told ourselves that nothing was happening yet, and there was no reason to panic. Then, when we received the call from Motol Hospital, saying we had to come for more tests, there was already some nervousness, but I still didn't allow myself to believe that something like this really concerned us. At that time, I still didn't fully understand what the disease (SCID) meant, how dangerous it was, or what the treatment involved, considering that AB appeared, at first glance, to be a perfectly healthy and content baby.
Initial management	
Immediately after the immune phenotyping, the family is advised to maintain strict home isolation and discontinue breastfeeding to prevent cytomegalovirus (CMV) transmission from the seropositive mother. Antibiotic prophylaxis with trimethoprim and immunoglobulin replacement are initiated. Human leukocyte antigen (HLA) typing is requested for the family, and lymphopoietic chimerism is excluded in the patient. All household contacts updated their vaccinations, where applicable. The family is offered psychological support.	From the moment we received the diagnosis, our whole world turned upside down in a second. These were the worst moments of our lives, mixing feelings of uncertainty, fear, confusion, hopelessness, absolute isolation from 1 day to the next, my hormones, and the fact that I had to immediately stop breastfeeding because of the CMV virus. I was on the brink of a breakdown. Our older children had just started to have colds the day we learned about the diagnosis, so we had to send them to their grandmother's, which was extremely hard. We were often separated for the first half of the year because they were frequently ill and, therefore, a danger to AB. We had imagined the first weeks would be beautiful, as they had been with our two older children. We are quite an active family and used to go on trips and vacations often, spent time outside, and met with friends, and all of this we had to stop abruptly.
Transplant and post-transplant period	
At 7 weeks, AB undergoes hematopoietic stem cells transplantation (HSCT) from his HLA-identical brother with alemtuzumab, fludarabine and treosulfan for conditioning and cyclosporine A and mycophenolate mofetil for graft-versus-host-diseases, (GVHD) prophylaxis. Platelet engraftment is documented on day 17 post-transplant and granulocyte engraftment on day 20. On day 21, symptoms of acute GVHD grade II appear and respond well to increased corticosteroid dosing, otherwise this period is uneventful. The patient is discharged from the hospital 30 days after HSCT.	The worst and longest part was waiting for the transplant. Although we were extremely lucky, that Matěj, our older son, turned out to be a suitable donor, which significantly sped up the process, the wait for the big day was very stressful. We were worried about AB staying in good condition and not catching an infection, and that Matěj would not get sick before the transplant. Then, when the day finally arrived, there was a slight relief for a moment because we had made it, and then the countdown began again until the first blood cells would start forming. Every morning, I waited impatiently for rounds, asking, “Any change yet?” This was also quite demanding. However, the staff at the transplant unit were great; every single one of them really tried to help and provide psychological support, which I appreciated very much. I knew he was in good hands.
Ongoing management and current status	
Initially, frequent viral respiratory infections were encountered, but no severe complications have occurred to date. Curiously, with the increasing proportion of autologous erythrocytes the cyanosis reappears. Two years post HSCT, AB is doing well, mixed chimerism is detected in unseparated peripheral blood, showing 80% recipient genotype, with donor-derived and functional T lymphocytes.	I dare to say that AB is doing well. He can now participate in practically all life activities without restrictions and do almost everything. Of course, I still have fears, and they will remain with me forever—when he's sick, how will his immune system handle it? I'm always tense about every illness but so far, he has cleared everything and showed us he is a very strong guy. At first glance, you wouldn't notice anything out of the ordinary—he is starting to talk, he is funny, he is smiling, and even when he's sick, he always has energy to spare and a smile on his face. We feel really lucky to have good doctors and health carers. In particular, we are grateful that everything was explained to us, and that all our (many) questions were being answered relentlessly throughout the process.

TABLE 3 (Continued)

On the NBS	
AB's parents kindly agreed to share their experience with the NBS programme and HSCT procedure with the families of other NBS-diagnosed SCID patients to come.	It was so good to know that thanks to the screening done right in the maternity ward, there is much more hope that everything will work out and AB will be cured. Since his SCID was discovered at such an early age, he thankfully didn't catch any infections and was able to undergo the transplant in the best possible condition.

early detection and intervention in severe actionable IEI. However, challenges such as false positives, appropriate prophylactic care and the most efficient HSCT protocols require ongoing international attention.

AUTHOR CONTRIBUTIONS

Markéta Bloomfield: Conceptualization; supervision; data curation; writing – original draft; writing – review and editing; investigation; project administration. **Eva Hlaváčková:** Writing – review and editing; investigation; data curation. **Helena Schneiderová:** Investigation; writing – review and editing; data curation. **Marek Turnovec:** Investigation; writing – review and editing. **Lukáš Tichý:** Investigation; writing – review and editing; data curation. **Zbyněk Čech:** Investigation; writing – review and editing. **Petr Chrastina:** Investigation; data curation; writing – review and editing. **Lenka Dvořáková:** Writing – review and editing; investigation; data curation. **Karolína Pešková:** Investigation; writing – review and editing; data curation. **Renata Formánková:** Investigation; writing – review and editing. **Petr Říha:** Investigation; writing – review and editing. **Marcela Vlková:** Investigation; writing – review and editing. **Petr Bejdák:** Investigation; writing – review and editing. **Magdaléna Havlišová:** Investigation; writing – review and editing. **Eva Froňková:** Investigation; writing – review and editing. **Tomáš Kalina:** Investigation; writing – review and editing; methodology. **Viktor Bíly:** Investigation; writing – review and editing; data curation. **Dita Říčná:** Investigation; writing – review and editing. **Hana Grombiříková:** Investigation; writing – review and editing. **Petr Sedláček:** Investigation; writing – review and editing. **Jiří Litzman:** Writing – review and editing; conceptualization; supervision. **Tomáš Freiberger:** Conceptualization; supervision; writing – review and editing. **Anna Šedivá:** Conceptualization; supervision; writing – review and editing; project administration. **Adam Klocperk:** Conceptualization; investigation; methodology; writing – original draft; writing – review and editing; data curation; supervision; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data is available from authors upon reasonable request. Material is not available.

ETHICS STATEMENT

The pilot study of SCID NBS in the Czech Republic was conceived and initiated as a nation-wide study by the Committee for SMA and SCID screening established by the Czech Ministry of Health and was performed in an established manner following the previous national pilot screening programmes, which were approved by the Ethical Committee of the National Screening Centre, Institute of Health Information and Statistics of the Czech Republic, Prague, Czechia. This study was designed as opt-in, with voluntary participation offered to all mothers giving birth from January 1st, 2022, to December 31st, 2023, in all neonatal wards in the Czech Republic. Dry blood spot (DBS) cards were collected upon collection of informed consent.

CONSENT TO PARTICIPATE

Written informed consent was obtained from all individual participants included in this study.

CONSENT TO PUBLISH

No identifiable information is published as part of this study.

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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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