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REVIEW

BENEFITS, CHALLENGES AND PROSPECTS OF NEWBORN SCREENING FOR PRIMARY IMMUNODEFICIENCY

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Abstract

More than ten years of implementation of newborn screening for severe combined immunodeficiency (SCID) using a T-cell receptor excision circle (TREC) and in recent years the TREC/ kappa-deleting recombination excision circle (KREC) assay have shown a number of advantages, which are not only the proper detection of SCID, to achieve better treatment outcomes and survival of children, as well as expanded knowledge on the prevalence of SCID, other diseases associated with T- and B-lymphopenia, but also allowed to detect new mutations associated with PID. The challenges include the inability to diagnose SCID with T-cell dysfunction and normal levels of T-lymphocytes and other SCIDs using the TREC assay, cytomegalovirus infection, that significantly worsens hematopoietic stem-cell transplantation prognosis (HSCT), economic issues, especially in low- and middle-income countries, and certain ethical issues. Considering the good worldwide experience, there are many reasons for a wider implementation of the newborn screening program for SCID in the world, including the Eastern Europe.

Keywords: severe combined immunodeficiency, diagnosis, newborn screening.

Résumé

Avantages, défis et perspectives du dépistage de l'immunodéficience primaire chez les nouveau-nés

Plus de dix ans de mise en œuvre du dépistage néonatal du déficit immunitaire combiné sévère (DIC) à l'aide de T-cell receptor excision circle (TREC), et ces dernières années le test TREC/ kappa-deleting recombination excision circle (KREC) ont montré un certain nombre de ses avantages, qui ne sont pas seulement une détection appropriée pour obtenir de meilleurs résultats thérapeutiques et la survie des enfants, ainsi que des connaissances élargies sur la prévalence du DIC, d'autres maladies associées à la lymphopénie T et B, ont permis de détecter de nouvelles mutations dans la PID. Les défis comprennent l'incapacité de diagnostiquer un DIC avec un dysfonctionnement des lymphocytes T et des niveaux normaux de lymphocytes T et certains autres DIC en utilisant la méthode TREC, une infection précoce à cytomegalovirus, qui aggrave considérablement le pronostic de la greffe de cellules souches hématopoiétiques (CSH), les problèmes économiques, en particulier dans les pays à revenu faible et intermédiaire, et certaines questions éthiques. Compte tenu de l'expérience mondiale positive, il existe de nombreuses raisons pour une mise en œuvre plus large

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List of abbreviations:
ADA – adenosine deaminase deficiency
A-T – ataxia-telangiectasia
BCG – Bacillus Calmette–Guérin
CHARGE syndrome – coloboma, heart defects, atresia choanae, growth retard genital abnormalities, ear abnormalities
CLOVES – congenital, lipomatous, overgrowth, vascular malformations, epidermal nevi, spinal / skeletal anomalies, and / or scoliosis
CMV – cytomegalovirus
DGS – Di George syndrome
DNA – deoxyribonucleic acid
EDA-ID – ectodermal dysplasia associated with immunodeficiency
ESID – European Society for Immunodeficiencies
HIV – human immunodeficiency virus
HSCT – hematopoietic stem-cell transplantation
KREC – kappa-deleting recombination excision circle
MHC – major histocompatibility complex
NBS – Nijmegen breakage syndrome
NGS – next-generation sequencing
PID – primary immunodeficiency
PCR – polymerase chain reaction
SCID – severe combined immunodeficiency
TREC – T-cell receptor excision circle
VUS – variants of uncertain sagnificance
WES – whole exome sequencing
WGS –whole genome sequencing

du programme de dépistage néonatal du DIC dans le monde, y compris en Europe de l'Est.

Mots-clés: immunodéficience combinée sévère, diagnostic, dépistage néonatal.

Introduction

Congenital pathology in children includes primary immunodeficiencies (PID), which lead to dysfunction of the immune system. The increased susceptibility to infections in children with PID is a serious problem, that can be fatal in the event of late diagnosis or inadequate treatment[1].

Among PIDs there is a group of severe combined immunodeficiencies (SCID), that manifest by severe bacterial and viral infections in infants during the first months of life[2-4]. According to the European Society for Immunodeficiencies (ESID) registry, in children with T-lymphopenia, the delay in diagnosis averages 3 months and ranges from 1 to 15 months, which may have adverse consequences for these children[5], as the effectiveness of hematopoietic stem cell transplantation (HSCT) in infants less than 3.5 months in the postnatal period is significantly higher (94%) than for older patients[6]. The results of our studies also confirm the delay in the diagnosis of PID, including SCID[7-8].

Therefore, the search for opportunities of early diagnosis of PID, especially SCID, has always been the focus of scientists. Due to the need for SCID diagnosis in the first months of life, there was a question of the possibility of neonatal screening. Newborn screening for SCID meets the general requirements formulated by Wilson and Jungner for screening programs, as these are rare conditions that cause serious health problems, they are not diagnosed by standard clinical examination, and are treatable, so their early diagnosis and treatment are important for achieving a positive result[9].

The immunological methods for the diagnosis of combined primary immunodeficiencies, in particular the determination of subpopulations of lymphocytes by flow-cytometry, are not performed at birth, because the child is born without signs of the disease. On the other hand, they cannot be used for routine screening, due to their complexity and cost[10-11]. Primary immunodeficiencies, actually SCIDs, can be diagnosed by determining the levels of T-cell receptor excision circle (TRECs) in a dry blood spot using the method of polymerase chain reaction (PCR) in real time[2]. TREC is a by-product of T-cell receptor gene recombination. Accordingly, low levels of lymphocytes

carrying these molecules in the peripheral blood prove T-cell lymphopenia[2-3]. The TREC method was developed more than 20 years ago[12]. Scientists have demonstrated that TRECs are specific for naive T-cells and have described their age-related decline in healthy people and in HIV infection[12]. In 2005, Puck and Chan proposed the use of this technique for population-based SCID screening[13].

The TREC method can detect only T-lymphopenia. However, there is another group of severe PIDs that are associated with B-lymphocyte deficiency, including X-linked agammaglobulinemia (Bruton's disease) and autosomal recessive hypogammaglobulinemia, which also lead to life-threatening conditions. In 2007, van Zelm et al developed a technique based on the detection of kappa-deleting recombination excision circle (KREC), using the PCR method[14]. KREC is an analogue of TREC, but is formed during the maturation of B-cells in the bone marrow.

In 2011 the effectiveness of the KREC for the diagnosis of B-lymphopenia in a dry drop of blood in a patient with X-linked agammaglobulinemia was demonstrated for the first time[15].

Newborn screening for PID

SCID detection

The main task of neonatal screening for PID is the early detection of SCID, before the development of infections, which allows to isolate such children in time and to carry out the necessary measures to prevent the development of opportunistic and other infections, to provide adequate treatment for children, which will improve their survival and quality of life[9].

In 2008, the newborn SCID screening program was first implemented in Wisconsin (USA)[16]. Today, newborn screening for SCID is implemented in all the USA states, Taiwan, Israel, New Zealand, some provinces of Canada, and Qatar[9]. Screening programs are widely supported by government and patient organizations, such as Jeffrey Modell Foundation (JMF), and Immunodeficiency Foundation (IDF)[17]. According to published data, in Europe the screening programs have been implemented in several provinces of Spain (Catalonia, Seville)[18], in Stockholm region (Sweden)[19], France[20], in several provinces of the Netherlands[3] and in Switzerland[21]. Pilot projects of newborn screening for SCID have been or are currently underway in a number of countries, in particular in Italy, Germany, Japan, Norway, Turkey, Slovenia, Iceland, Denmark, Poland, the Czech Republic and Brazil[9,22]. The Konya Declaration has stated the need to implement newborn screening for PIDs to improve the diagnosis of PIDs in the J-Project countries, which incorporate Eastern and Central Europe[23]. The first population screening study for identification of SCID in Central and Eastern Europe was conducted in the Polish-German transborder area of West Pomerania, Mecklenburg-Western Pomerania, and Brandenburg, in collaboration with two centers from Warsaw in 2017[24].

The use of TREC assay for SCID newborn screening in USA revealed 52 cases in 11 states between 2009 and 2013[25] and 50 cases of SCID between 2010 and 2017 in one state of California[26], which saved dozens of lives and gave them a chance for a quality life.

The introduction of a screening survey in Switzerland in January 2019, using a combined analysis of TREC and KREC, led not only to a better clinical outcome using allogeneic hematopoietic stem-cell transplantation (HSCT) or gene therapy in children with SCID, but also to improved patient's management with a help of the multidisciplinary team consisting of obstetricians and gynecologists, neonatologists, pediatricians, medical geneticists, pediatric infectiologists and immunologists, specialized nurses, psychologists and social workers[21].

Establishing the real prevalence of SCID

Screening also made it possible to establish the real prevalence of SCID in different populations. Studies have shown that the prevalence of SCID is significantly higher than shown by pre-screening statistics[9,24]. Thus, in the USA, the prevalence of SCID before screening was 1 in 100,000 newborns, and the analysis of screening programs in 11 states showed an average prevalence of SCID almost twice as high (1 in 58,000 newborns)[25]. Overall, according to published data, the prevalence of SCID ranged from 1 in 22,374 in Israel[27] to 1 in 130,900 in Spain (Catalonia)[18] and 1 in 131,485 in Taiwan[28].

TREC or TREC/ KREC

In most countries, the populational SCID screening program uses the TREC analysis[3,16,18,19,25-28]. TREC/ KREC assay was used in Sweden[19], and since 2019 it has been used in Switzerland[21]. TREC/ KREC assay has also been used in a number of pilot studies in Spain (Seville)[29], Iran[30], and in the Polish-German transborder area[24].

The use of simultaneous TREC/ KREC analysis has a number of advantages over using TREC alone. The combination of techniques allows to detect not only congenital B-cell defects, but also other forms of PID, which can be missed by TREC analysis, in particular, late onset of adenosine deaminase deficiency (ADA), Nijmegen breakage syndrome (NBS) and other conditions[31]. The use of TREC/ KREC

assay in countries with a high incidence of these diseases may be particularly important. This also applies to Ukraine, where there is a high prevalence of the Slavic mutation of NBS and agammaglobulinemia[32]. However, the question of the conformity of these PID diagnoses to the general principles of neonatal screening still needs to be studied.

Related findings

In addition to the detection of SCID, the TREC/ KREC assay allows to detect other conditions with T- and B-lymphopenia. The frequency of detected lymphopenias, according to studies, ranged from 1 in 920 subjects in a study in Sweden[33] to 1 in 7300 subjects in the USA[25].

However, as shown by the results of screening programs, low levels of TREC/ KREC are detected not only in SCID, but also in other immunodeficiencies and diseases that run with low levels of T or B cells, in particular in Di George syndrome (22q11 deletion syndrome), ataxia-telangiectasia (AT), DOCK8 deficiency, Kabuki syndrome, ectodermal dysplasia associated with immunodeficiency (EDA-ID), NBS, CHARGE syndrome (coloboma, heart defects, atresia choanae, growth retard genital abnormalities, ear abnormalities), cartilage-hair hypoplasia, CLOVES (congenital, lipomatous, overgrowth, vascular malformations, epidermal nevi, spinal/ skeletal anomalies, and/or scoliosis), Rac2 deficiency[9].

Individual chromosomal abnormalities are also characterized by T-lymphopenia and can be detected with the TREC assay. In particular, children with Noonan's syndrome, Jacobsen's syndrome, Fryns syndrome, Renpenning's syndrome and other cytogenetic abnormalities also show low levels of TREC[9].

Auxiliary diagnosis of a number of diseases using the TREC/ KREC assay has its positive sides, as a number of immunodeficiencies may manifest in the second – third year of life. Low awareness of physicians regarding PID, vagueness of the onset of clinical symptoms lead to a late diagnosis of PID, which can have certain negative consequences and worsen the quality of life[34-36]. At the same time, the early diagnosis of PIDs such as A-T, NBS, DGS, DOCK8 deficiency can have advantages, as it will allow proper preventive measures. In particular, limited use of X-rays, which can cause the early development of oncopathology in children with impaired DNA repair, can prolong the life of these patients. This is important for Ukraine, especially its western regions, where there is a high frequency of these PIDs[32].

In addition, conditions as prematurity, certain maternal diseases during pregnancy, immunosuppressive drugs etc, may also be accompanied by T-lymphopenia and low TREC levels will be detected.

Detection of new mutations

With the help of population-based newborn screening for SCID, it was possible to detect a number of new mutations that are accompanied by T-lymphopenia, as well as to obtain new information about T-cell developmental pathways[26]. Thus, the screening in California has identified new mutations in *BCL11B* and *EXTL3* that are associated with T-cell deficiency[26]. The detection of new mutations has been reported in other studies[18,25,27-28].

BCG vaccination

Some countries corrected the time of Bacillus Calmette–Guérin (BCG) vaccination after implementation of population screening for SCID. Thus, in Taiwan, the time of BCG vaccination was postponed until the results of screening, which allowed to increase the percentage of vaccinated newborn children at the age of 1-5 months from 18% in 2010 to 46.9% in 2014, without increasing the incidence of tuberculous meningitis[28].

Challenges of newborn screening for PID

Need of cut-off value for urgent positive result and other positive results

Detecting a wide range of diseases and conditions using the TREC/ KREC assay can have a number of disadvantages, which are associated with both additional economic costs and increased parental anxiety with false-positive results. Therefore, a very important issue is to establish a cut-off value for the detection of SCID, other PID and conditions accompanied by T- and B-lymphopenia, which requires pilot projects before the introduction of large-scale screening.

Missed cases

A limitation of the TREC assay is the inability to diagnose some SCID, in particular functional disorders of the T-cell immunity, late onset of ADA deficiency, Zap70 deficiency, major histocompatibility complex (MHC) class II deficiency[9]. Several SCIDs have been missed by screening in California, including MHC class II deficiency, prompting scientists to look for methods that would cover the diagnosis of a wider range of PIDs[26]. Thus, a pilot study of newborn screening for SCID in Italy used the method of determining TREC and mass spectrometry to determine ADA deficiency[37].

Cytomegalovirus infection (CMV)

CMV infection remains a serious problem. Thus, two dead children with SCID in California who were detected by screening were diagnosed with

Table 1. Benefits and challenges of newborn screening for SCID.

Benefit	*Challenge*
Early detection of SCID	Missed cases (inability of the TREC analysis to detect SCID with T-lymphocytes dysfunction without reducing the number of T cells)
	The need for management and long-term follow-up of children diagnosed with SCID
Early isolation of children with suspected SCID to avoid infections	Difficulties in preventing CMV infection
Secondary findings: detection of other diseases with T- and B-lymphopenia	Additional economic costs, increased parental anxiety with false-positive results, the need to define clear cut-off values for the diagnosis of SCID and other diseases
Detection of new mutations	Possible VUSs that are not related to the disease
Establishing the real prevalence of SCID	The need to increase HSCT, increase transplant units, which can be a problem in low- and middle-income countries
Possibility to choose TREC or TREC/ KREC assay	The need for a pilot study and analysis of the prevalence of different types of PID, which will allow to choose the most appropriate and cost-effective method
BCG vaccination can be postponed until screening results are obtained to avoid disseminated infection in children with SCID	Possible increase in the incidence of tuberculous meningitis

CMV infection and in one of them CMV was the direct cause of death[26]. Even precautions, such as isolation etc, could not prevent CMV exposure through vaginal discharge or breast milk, if the mother had a previous infection[26], which requires further improvement in CMV prevention measures.

In Switzerland, recommendations have been developed that include examination of mothers for CMV and features of breast-feeding in the case of abnormal TREC results[21]. Therefore, the management depends on the level of TREC, gestational age and CMV status of the woman.

Additional costs

Economic feasibility is also always considered in the context of the implementation of screening programs[17,20]. Neonatal screening should have not only clinical feasibility, but also be cost-effective. On the other hand, nothing can be compared to dozens of lives saved. Economic costs take into account not only the funds for tests, but also additional funds for equipment, wages, further diagnosis[13,20]. However, studies show a reduction in the cost of treatment of the patient in the case of timely diagnosis[17]. In addition, the patient has a better chance of a higher quality of life, which can also reduce the cost of screening[13,17].

Ethical issues

Ethical issues are related to both obtaining parental consent and tactics of informing parents in the case of a positive result. Consent for screening examinations in most countries is obtained through an 'opt out' process[22]. However, the storage of genetic materials and the impossibility of its use for other purposes must also be taken into account.

An important issue is the choice of tactics to inform parents about the positive results of screening. Studies in the United States have shown that families with children with SCID diagnosed by newborn screening experience certain emotional and other challenges at various stages of diagnosis and treatment, from screening and diagnosis to treatment options and prolonged isolation[38].

In Table 1, we summarize the achievements and challenges of newborn screening for SCID that are known today.

Prospects of newborn screening for PID

Each year, newborn screening programs for SCID spread to new countries and continents, including middle-income countries. Prospects for the development of newborn screening for PID are seen in several directions.

On the one hand, measures should be aimed at improving the support of children diagnosed with SCID. Recommendations for diagnostic follow-up investigations, measures to reduce the risk of CMV infection, vaccination and management are being developed and improved[21]. Given the need of informational and emotional support for families in which children have been diagnosed with SCID through newborn screening, the implementation of SCID

Compass web site was proposed, which contains useful and important information that can help parents overcome stress and uncertainty at different stages of diagnosis and treatment[38].

On the other hand, PID is a large group of diseases, which currently numbers more than 450 diseases[1]. Many of them can also be severe, life-threatening, and the only reliable treatment for some of them may be HSCT or gene therapy. In particular, diseases of the complement deficiency, phagocyte abnormalities, innate immune defects often have an atypical course, which leads to late diagnosis[8], which encourages scientists to seek methods for early diagnosis of these diseases[22] . The use of other screening methods based on protein determination is proposed as a method of identifying newborns with complement disorders and granulocytes, which will allow early interventions for prevention of potentially dangerous consequences[39].

Considering the rapid development of genetics and the declining cost for genetic testing in recent years, the use of whole exome sequencing (WES) and genome sequencing (WGS) in the diagnosis of congenital diseases, including PID, is increasingly being considered[9,40-41]. The use of these methods in neonatal intensive care units is considered to be especially promising[41]. Therefore, the future use of genome or exome sequencing in neonatal screening, including for PID identification, looks promising today.

Variants of uncertain sagnificance (VUS) are common in NGS analyzes, which requires the development of a clear plan for the detection and reporting of such results. In addition, the detection of «unexpected» or unrelated genetic mutations can have far-reaching consequences for humans and their families. Therefore, it seems more appropriate to detect mutations in genes that are known to lead to serious disease or reduced life expectancy, and for which there is a cure. The use of WES and WGS for screening requires addressing both economic and ethical aspects[9].

Meanwhile, there are lots of diseases which currently have no effective treatments and lead to fatal consequences in the first years of life[40]. Therefore, prenatal diagnosis in families with a history of PID remains an important area[42].

Prospects in Ukraine

Neonatal screening in Ukraine is currently performed for 4 diseases: phenylketonuria, congenital hypothyroidism, cystic fibrosis and adrenogenital syndrome. Considering the growth of SCID cases in recent years and the development of HSCT in Ukraine, including from a non-family donor, there are prospects for the implementation of newborn screening for SCID by TREC or TREC/ KREC assay. In 2020, the Ukrainian Association of Pediatric Immunology initiated the introduction of newborn screening for SCID in Ukraine.

Conclusions

More than ten years of implementation of newborn screening for SCID in the world using TREC, and in recent years the TREC/ KREC method have shown a number of advantages, which are not only the proper detection of SCID, that helped to achieve better treatment outcomes and survival of children, as well as expanded knowledge on the prevalence of SCID, other diseases associated with T- and B-lymphopenia, allowed to detect new mutations associated with PID.

Challenges include the inability to diagnose SCIDs with T-cell dysfunction and normal T-lymphocyte levels, as well as other SCIDs using the TREC method, CMV infection, which significantly worsens the prognosis of successful HSCT, economic issues, especially in low and middle income counties and certain ethical issues.

Due to the positive experience in many countries, there are many reasons for a wider implementation of the newborn screening for SCID in the world.

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O.B., T.H. wrote the manuscript, M.K., N.Y., O.B., T.H. were responsible for the collection and assembly of the articles/ published data, and their inclusion and interpretation in this review. All authors have read and agreed to the published version of the manuscript.

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