Newborn Screening for Severe Combined Immunodeficiency in Israel

Erez Rechavi¹, Atar Lev¹, Talia Saraf-Levy², Amos Etzioni³, Shlomo Almashanu²,*† and Raz Somech¹,*†

¹ Pediatric Department A and the Immunology Service, "Edmond and Lily Safra" Children's Hospital, Jeffrey Modell Foundation Center, "Sackler" School of Medicine, Tel Aviv University, Tel-HaShomer, 52621, Ramat Gan, Israel.
² The National Center for Newborn Screening, Ministry of Health, Tel-HaShomer, 52621, Ramat Gan, Israel.
³ Ruth Children Hospital, Rappaport Faculty of Medicine, Technion, 3200003, Haifa, Israel.
† Shlomo Almashanu and Raz Somech contributed equally to this article and are equal corresponded.
* Correspondence: raz.somech@sheba.health.gov.il (R.S.); Shlomo.Almashanu@sheba.health.gov.il (S.A.). Phone/Fax: +972-3-5303477.

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Abstract: Newborn screening (NBS) programs for severe combined immunodeficiency (SCID), the most severe type of primary immunodeficiency, are being implemented in more and more countries with every passing year. Since October 2015, SCID screening via T cell receptor excision circle (TREC) quantification in dried blood spots (DBS) has been part of the Israeli NBS program. As an NBS program in its infancy, SCID screening is still evolving, making gathering input from the various programs crucial for compiling an ideal screening algorithm. The relatively high rate of consanguineous marriages in Israel, especially among non-Jews, correlates with an increased incidence of SCID. The Israeli algorithm uses a commercial kit and consists of a two-Guthrie card confirmation system prior to referral to a national immunology center. Preliminary data from the first year and a half of SCID screening in Israel has identified a surprisingly high prevalence of DNA cross-link repair protein 1c (DCLRE1C; ARTEMIS) mutations as the cause of SCID in Israel. The clinically unbiased nature of SCID screening helps unearth mild/leaky SCID phenotypes, resulting in a better understanding of true SCID prevalence and etiology.

Keywords: newborn screening (NBS); severe combined immunodeficiency (SCID); Israel; ARTEMIS; DNA cross-link repair protein 1c (DCLRE1C); T cell receptor excision circle (TREC).

1. Introduction

Annually, there are approximately 185,000 live births in Israel. Approximately 75% of these newborns are Jewish, with the remaining 25% being of Muslim Arab, Christian Arab and Druze origin [1]. Consanguineous marriages are relatively common in Israel in general, but in particular among non-Jews, with a preference for first cousin marriages. While the rate of consanguineous marriages among all Israeli populations is in steady and significant decline, according to Na'amnih et al, almost a quarter of all non-Jews in Israel still marry within the extended family [2]. This naturally leads to an increased rate of recessive Mendelian disorders in Israel compared with other Western countries, contributing significantly to infant morbidity and mortality, especially among non-Jewish families [3]. Primary immunodeficiencies are no exception to this rule [4]. In a recent study conducted in southern Israel, Broides et al found an incidence rate of 55.8:100,000 for all different types of typically primary immunodeficiencies in the Bedouin (Muslim Arab) population [5]. In light of this alarmingly high incidence, preventive and early diagnostic measures have been

put in place in Israel to improve the outcomes of children suffering from the most serious form of primary immunodeficiency, severe combined immunodeficiency (SCID).

Carrier genetic screening for several SCID causing genes is available for all Israeli populations and is free of charge for populations with increased risk, like Bedouins from the south of Israel [6]. Since October 1, 2015, SCID screening has been incorporated into the nationwide Israeli newborn screening (NBS) program. In general, prior to 2008, the Israeli NBS program included phenylketonuria and congenital hypothyroidism, however, after this time it was extended to include many of the relatively common, treatable congenital diseases in Israel, including congenital adrenal hyperplasia, maple syrup urine disease, homocystinuria, tyrosinemia, methylmalonic acidemia, propionic acidemia, glutaric aciduria, and medium- and very-long-chain acyl-CoA dehydrogenase deficiency. Swift detection of infants afflicted with one of these illnesses through the NBS program allows for early, disease course-altering treatment, and has significantly reduced associated morbidity and mortality [7].

2. History of the Israeli SCID screening

SCID includes a heterogeneous group of genetic disorders characterized by profound deficiencies of T cells, and in some of the variants B and/or Natural Killer cells, numbers or function. If untreated, infants with SCID succumb early in life to severe and recurrent infections [8]. Recent estimates, based on the results of American SCID neonatal screening, indicate an SCID incidence of 1.7:100,000 in the general population [9], but it is substantially higher in some populations. In the aforementioned Broides et al study, the overall incidence of SCID/CID in the Bedouin population of southern Israel was 18.3:100,000 [5].

Given that children with SCID have a much higher survival rate if diagnosed early (through screening or positive family history) [10], and shortly after the publication of successful results from the first SCID neonatal screening in Wisconsin back in 2008, we began advocating for the inclusion of SCID in the Israeli NBS program using quantification of T cell receptor excision circles (TREC) [11]. This test, when performed in peripheral blood of suspected patients, is routinely used by us and others to successfully diagnose patients with significant T cell immunodeficiency [12–14], to appreciate the depth of the immunodeficiency associated with syndromes such as ataxia telangiectasia [15] or DiGeorge syndrome [16], to follow immune reconstitution after bone marrow transplantations [17], to measure the activity of autoimmune diseases [18] and even to monitor T cell development during fetal life [19]. In 2011, we performed a pilot study to provide a proof of concept for detection of seven known SCID patients (compared to 15 healthy controls) through quantification of TREC in Guthrie cards stored in the Israeli NBS bank. All patients were diagnosed on clinical basis. The first features suggestive of SCID presented at age 3.1 ‒ 2.4 months. Nevertheless, the diagnosis was made only 4.1 ‒ 2.9 months later. TREC copies in peripheral blood samples were undetectable or significantly low at clinical diagnosis. Next, TREC was retrospectively quantified by real-time Polymerase Chain Reaction (rtPCR) in dried blood spots (DBS) from Guthrie cards obtained immediately after birth. TREC copy numbers for all controls were above the threshold of 30/3 mm punched disk (average 85.5 TREC copy numbers/3 mm punched disk, range 35–212 copies), whereas all seven SCID patients were below the threshold (six undetectable, one, with complete DiGeorge phenotype, remarkably low) [20]. In January 2014 we issued a call to add SCID screening to the mandatory, government subsidized national NBS program in Israel, wherein we established the necessity for SCID screening in Israel and quantification of TREC as a specific, sensitive and cost-effective tool for detection of T cell lymphopenia, including SCID [21]. On December 30, 2014, the Israeli Committee for the Expansion of Medical Services submitted its recommendation to incorporate SCID screening via TREC into the NBS program to the Ministry of Health, and on October 1, 2015, Israeli SCID screening was officially launched [6]. Our pilot study [12] was recently included in a systematic review which tried to characterize the TREC-based NBS for SCID [22]. Thirteen studies were included, re-confirming known SCID cases and reporting new SCID cases in more than 3 million newborns. The sensitivity for typical SCID reached 100% and SCID incidence was ~1.7:100,000. The authors reported on retesting, repeat DBS, referral rate and
positive predictive value of the test. They suggested that adopting modified screening algorithms for preterm/ill infants would reduce the number of false positive test results [22].

3. Israeli SCID screening algorithm

The Israeli SCID NBS program uses the commercial EnLite™ Neonatal TREC kit (Wallac Oy, Mustionkatu 6, FI-20750 Turku, Finland). DBS punches of 1.5 mm diameter are inserted into a black, 96-well PCR plate. DNA is eluted without extraction. Unlike extraction, which requires precipitation and cleaning of DNA, elution (breaking cells and nuclei with heat) does not require transferring the sample from one test tube to another, thus avoiding unnecessary DNA loss. Next, PCR amplification of TREC and beta-actin, an internal control for each specimen, is performed. Four PCR plates are processed in parallel, each plate containing a standard TREC curve in triplicates, as well as positive and negative controls for both targets. The manufacturer reports 100% sensitivity for typical SCID in a pilot study analyzing TREC in DBS from 5,000 healthy and 18 SCID-affected neonates [23]. With the current cut-off of 23, the specificity in our cohort is 99.7%. With the original TREC cut-off of 36, the initial positive result rate was 4.24%. The threshold was then lowered to 28 (1.84% positive result rate) and finally 23, the current cut-off, which produces overall 0.95% positive results on the first test (in early preterms, a lower cut-off is used de-facto for determining the need for retesting). Retesting consists of two additional punches taken from different DBS of the same, initial Guthrie card. If both are below cut-off for TREC with normal amplification of beta-actin, a second, confirmation Guthrie card is obtained and tested in duplicate. Only in the event of all five tests returning positive is the child referred for validation at the national clinic and laboratory. Immunological validation and workup consists of retesting for TREC in peripheral blood, lymphocyte subset determination via flow cytometry and, if necessary, T cell receptor repertoire analysis by flow cytometry and lymphocyte proliferation assay.

The Israeli presumptive positive algorithm diverges from the standard approach at several junctures. First, the proposed Enlite™ kit algorithm considers a positive result in either of the duplicate DBS tests as cause for further validation, whereas we proceed with evaluation only if both are positive (once normal—always normal). Second, other SCID screening programs, including California, where the Enlite™ kit is also employed, skip the second Guthrie card validation step and proceed directly to referral to an immunologist. We maintain that the relatively high rate of initial positive results, of which only a small fraction are true positives, warrants this additional verification measure prior to referral to an immunologist.

Preterm neonates are more likely to produce false positive (FP) results [24]. As such, so long as a preterm infant is hospitalized, a new, repeat Guthrie card is tested every two weeks. Only preterm infants whose results are consistently positive (fail to normalize with age) are referred to immunologist verification upon discharge from the hospital.

Overall, 290,864 neonates were screened for SCID between October 1, 2015 and April 30, 2017. With the double verification process, fewer than 50 patients were referred for immunological workup.

4. Screening reveals increased prevalence of ARTEMIS mutations

The relative frequency of different forms of SCID varies in different countries and ethnic groups. The most common SCID phenotype in the United States (and presumably worldwide) is T-B+NK-, caused in most instances by a mutation in Interleukin 2 Receptor γ Chain (IL2RG, X-linked SCID) [8]. In contrast, the most frequent type of SCID phenotype in Israel is autosomal-recessive T-B-, whereas X-linked SCID is rare [25]. Prior to initiation of the Israeli SCID screening program, the majority of Israeli SCID patients were thought to harbor mutations in either Recombination Activating Gene 1/2 (RAG1 or RAG2). However, with the advent of the screening, a host of autosomal recessive, T-B-SCID patients have been diagnosed with mutations in the DNA cross-link repair protein 1c (DCLRE1C) gene, termed ARTEMIS.

ARTEMIS is a member of the metallo-β-lactamase superfamily and, like RAG1/2, is part of the somatic recombination machinery. Briefly, after the RAG complex induces double-strand breaks in
the T cell receptor and immunoglobulin genes, the blunt edges of the chromosomes are closed to form a hairpin structure. ARTEMIS then opens this hairpin to allow for joining of the selected V (D)J segments [26]. Historically, ARTEMIS mutations are very rare compared to other causes of SCID [8]. This could result from under diagnosis due to the great phenotypic variability induced by ARTEMIS mutations, with some mutations correlating to leaky SCID or combined immunodeficiency (CID) presentations [27]. These milder phenotypes, less likely to warrant a thorough genetic evaluation, have only recently been diagnosed as ARTEMIS variants of SCID, in most cases through whole exome sequencing. A similar shift in SCID genotypes has been observed in the United States following implementation of SCID screening, from the aforementioned dominance of severe X-linked SCID towards more autosomal-recessive, milder variants of SCID [28].

The NBS program, unbiased by clinical presentation, thus allows for the early detection of these, previously overlooked, children.

Despite its rarity in the general population, T-B-SCID due to ARTEMIS mutations has a very high incidence among Athabaskan-speaking Navajo and Apache Native Americans (termed Athabaskan-SCID), with a common nonsense founder mutation [29]. A similar founder effect is apparent in the Israeli Muslim Arab population, with an increased frequency of ARTEMIS and a common insertion mutation shared by several patients from families of no immediate ancestral proximity.

The definitive treatment for all SCID patients is hematopoietic stem cell transplantation (HSCT), with broad antibiotic and antifungal treatment as a bridge from diagnosis to transplantation. However, finding the causative gene can lead to critical adjustments in management. Because ARTEMIS is part of the cell’s DNA repair machinery, it is imperative to use reduced-intensity HSCT protocols for these patients.

As T-B-SCID is the prevalent form of SCID in Israel, whether due to ARTEMIS or RAG mutations, it stands to reason that screening for B cell deficiency (via measurement of the Kappa-deleting receptor excision circle, KREC, alongside TREC) would amplify the sensitivity of SCID screening in Israel, while also allowing for the detection of exclusively B cell deficiencies [30].

5. Screening limitations

While the TREC-based assay for neonatal SCID detection is highly effective, it is not without limitations. Variability in TREC levels at birth, especially among preterm infants, yields a significantly higher FP rate for SCID NBS than for other screened diseases. Some of this may be attributed to the normal growing pains of a technique still in its infancy, and retesting from separate Guthrie card punches eliminates most falsely-flagged newborns with ease, and without troubling the infant's family. In Israel, gradual lowering of the threshold for retesting has reduced the FP rate substantially, while, to our knowledge, no SCID patient had gone undetected in Israel since the initiation of the screening. With time, and experience, the FP rate should reach satisfactory levels.

A more difficult challenge, and one that is unlikely to be as easily resolved, is the inherent inability of the TREC assay to detect immunodeficiencies that result from T cell dysfunction without T cell lymphopenia [31]. These include patients with certain mutations such as CD40 Ligand (CD40L), Interleukin 10 Receptor (IL10R) and Forkhead Box P3 (FOXP3) [32]. A prime example for this challenge is Major Histocompatibility Complex class II (MHC-II) deficiency, a variant of SCID in which the development and function of CD4 T cells is impaired. While T cell function is severely impaired, resulting in typical SCID susceptibility to infection, lymphocyte counts are normal. Subsequently, TREC copy numbers in DBS from confirmed MHC-II are similar to those of control patients and significantly higher than TREC levels for other forms of SCID [33]. In California, where SCID screening began in August 2010, several children with severe forms of immunodeficiency, including MHC-II, had been missed by the screening due to normal TREC levels at birth [31]. As SCID NBS programs, including ours, grow older, more and more of these normal TREC SCID variants are bound to be falsely cleared by the screening. The worldwide implementation of neonatal screening for SCID is a game-changing development in the diagnosis and treatment of
immunodeficiency, but there is no replacement for clinical suspicion, and there is always room for improvement.

6. Conclusion

TREC based assay has proven to be a sensitive and efficient method for SCID detection, as well as other cellular primary immunodeficiencies. Moreover, its quantification in dried blood spots obtained at birth permits population-based newborn screening. Furthermore, its unbiased nature allows for the detection of less severe SCID variants and will enable us to accurately determine the true prevalence of SCID. On the heels of the success of SCID screening, we are hopeful that NBS programs for additional primary immunodeficiencies will soon follow suit, with screening programs for B cell immunodeficiencies, Neutrophil immunodeficiencies such as Chronic Granulomatous Disease and genetic syndromes with a component of immunodeficiency like DiGeorge Syndrome. We believe that with the implementation of NBS for SCID, a disease once considered universally fatal, close to 100% of children with SCID will receive early treatment and a substantially better chance at a cure.

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