

Screening for severe combined immunodeficiency in newborns

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This issue of the *Journal* is devoted to the critical concept of screening for severe combined immunodeficiency (SCID), a long-sought goal of clinical immunologists engaged in hematopoietic stem cell transplantation (HSCT) for SCID, a congenital disease of many causes that is fatal in the first 1 to 2 years of life.¹ The clinical immunologists chosen for the reviews in this thematic issue were selected for their expertise in the diagnosis and management of infants with SCID, their leadership in devising methods of early diagnosis of SCID, and their ability in implementing the current method of detecting a low number of T-cell receptor excision circles (TRECs) in the blood of neonates.²⁻⁴

Rebecca Buckley's clinical review⁴ presents an overall view of the long quest for a screening test for SCID. It is an excellent account of how investigators arrived at the use of the TREC assay to detect SCID (low T-cell count) in infants with immune deficiency who are threatened by opportunistic infections. One of the early tests proposed to alert pediatricians to problems of SCID was the absolute lymphocyte count. Here, pediatricians had to remember the temporary lymphocytosis in the neonatal period that provides newborns with approximately 3 times the number of lymphocytes seen in older children. Excellent age-adjusted control data on healthy children from birth to 18 years of age are now available.⁵ The 10th and 90th percentiles of normal lymphocyte values are 3400 and 7000 cells/ μ L in the first 3 months of life. Therefore a value of 1000 lymphocytes/ μ L, which is the normal adult mean average, does not apply to neonates and should serve as an alarm for repeat testing or referral to an immunologist for further evaluation.⁶ Because neonates with SCID are usually healthy at birth because of maternal antibody protection, obtaining a complete blood count and absolute lymphocyte count would require justification and would be costly. The proposal by Chan

and Puck² to use dried bloodspots collected on the well-known Guthrie card, which is used for screening of metabolic defects, became the favored choice for SCID detection. Dr Buckley relates the persistent path of seeking approval for the TREC screening test for SCID through various federal health agencies beginning in 2005 and the final approval and recommendation of Dr Kathleen Sibelius, the Secretary of the Department of Health and Human Services in 2010. By May 2011, almost one million TREC screening assays had detected 14 cases of SCID, 6 cases of SCID variants, and 40 infants with various forms of T-cell lymphopenia in the 6 states and the 1 US territory performing these tests on a routine basis. All of the infants with SCID were rescued from certain death by using HSCT.

Jennifer Puck⁷ provides us with a molecular mechanisms article that defines the rearrangements of the T-cell receptor gene in neonates that creates the circular TREC structures that can be measured to detect a lack of T lymphocytes in the various causes of SCID and some combined immunodeficiencies.⁸ She describes the important difference in a screening test for SCID performed on a large scale at centralized state public health laboratories and the targeted individualized testing in immunology laboratories for a child with suspected SCID. The former testing must use small amounts of preferably dried blood on filter paper and lends itself to rapid implementation by using high-throughput technology at a low cost. The latter testing requires fresh blood with viable cells and by definition is very expensive due to the required sophisticated technology and expertise of those persons performing the testing. Although several screening tests for SCID have been evaluated, because of the lability of certain proteins being measured and the high rate of false-negative results, these tests have left the TREC assay as the screening method of choice. The illustrations in Puck's article are particularly instructive in understanding how TRECs are made from the rearrangements of the T-cell receptor DNA locus and how measurements of TREC levels yield a diagnosis of T-cell cytopenia. Also included in this article is a tabular summary of the results of performing the TREC assay on greater than 500,000 newborns: 900 DNA samples (<0.08%) were amplification failures requiring a second heel stick (failure rate similar to other newborn screening); of these 900, 504 were from neonates weighing less than 1500 g, and the remainder were obtained in an inappropriate manner. There were 50 positive TREC assay results (ie, low number), with 20 having low T-cell numbers confirmed by means of flow cytometry or gene sequencing. The final diagnosis of primary immunodeficiencies of these children were 6 cases of SCID (2 with IL-2 receptor γ deficiency, 2 with IL-7 receptor α deficiency, and 2 with recombination-activating gene 1 deficiency), 2 SCID variants, 1 case of Omenn syndrome, 3 cases of complete DiGeorge anomalies, and 1 case of trisomy 21. There were also 6 infants with a diagnosis of secondary T-cell lymphocytopenia that were due to gastroschisis

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($n = 2$), gastrointestinal atresia ($n = 1$), and prematurity ($n = 3$). These data strongly suggest that the incidence of SCID and other serious T-cell deficiencies is much higher than what has been taught for the past 4 decades (1/100,000); the true incidence is approximately 1/40,000. These estimates will become more certain as more states come online with screening infants for SCID with the TREC assay.

Dr Puck concludes her review⁷ with a peek into the future of early testing of newborns for SCID by predicting that deep gene sequencing (whole exome or whole genome) might become the standard of laboratory diagnosis. In this manner non-T-cell deficiencies would be detected. In this direction the recent report on using κ chain receptor excision circles offers the hope of detecting neonatal B-cell deficiencies.⁹

In a Current perspectives article Verbsky et al¹⁰ have given us the results of the first TREC screening program in the United States, the state of Wisconsin's experience. From the beginning of the time the TREC assay was used for detection of patients with SCID in Wisconsin, 5 patients with SCID have been identified and referred to immunologists for definitive therapy with hematopoietic stem cells. In a 5-year experience, a false-positive rate of 0.018% was recorded with a specificity of 99.98% in more than 200,000 infants screened. Seventy-two infants had low TREC values (T-cell lymphopenia) from non-SCID factors, including anatomic lymphatic abnormalities, chromosomal abnormalities, and metabolic abnormalities. The 5 cases of SCID were due to 1 RAC-2, 1 adenosine deaminase, 1 $T^+B^-NK^+$, and 2 $T^+B^+NK^+$ deficiencies. This early experience clearly demonstrates the absolute need to have TREC data interpreted and further evaluated by an immunologist well versed in the diagnosis and management of SCID and related T-cell deficiencies. Follow-up testing of the specific causes of SCID is essential. Eventually, design of a technology that will directly identify gene mutations on the dried bloodspots currently used for the TREC assay will be a more direct approach to the most rapid mode of diagnosis, but the hundreds of mutations in the various SCID conditions and the constant discovery of new mutations at the moment preclude this direct screening assessment of specific immunodeficiencies.

This urgent need to quickly identify infants with SCID is rooted in the experience of every immunologist who is confronted with patients with SCID who are late in coming to diagnosis because of several reasons, such as lack of awareness of what constitutes SCID; acquisition of serious infection; and failure to thrive and inadvertent live viral vaccine exposure.¹¹ A family perspective survey has reinforced these findings,¹² and a single-family vignette of the first child with SCID born in a rural community with rudimentary health care and perishing compared with the second child with SCID coming to diagnosis by 5 days of life and receiving a successful transplantation at 5 weeks of life illustrate the power of early diagnosis.¹³ The first child was not given a diagnosis until she was 10 months of age, and by that time, she had systemic infection with several chronic viruses and recurrent bacterial infections. She succumbed to infection at 13 months of age despite attempts to reconstitute her immune system with HSCT. The second child was brought to a clinical immunologist as a newborn and was given a diagnosis of CD3 δ SCID (same mutation as his sister). He was given an HSCT from a 6/6 HLA-matched cord blood donor with successful engraftment and immunoreconstitution.

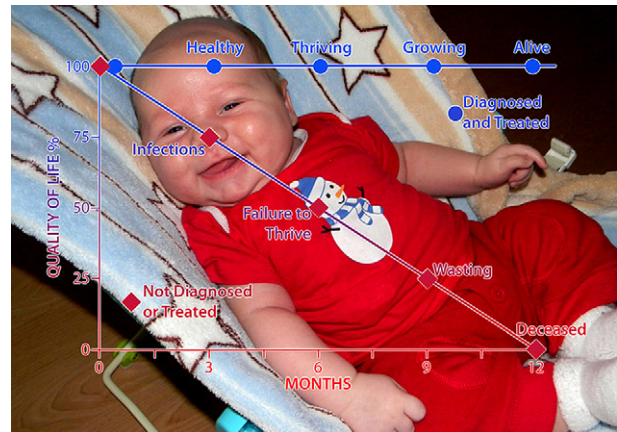


FIG 1. Five-week-old male child with CD3 δ SCID just before HSCT. Superimposed on the figure is a graphic illustration of the early lives of infants with SCID, both those with and those without early diagnosis and definitive treatment.

The cover of this issue of the *Journal* contains the picture of this healthy-appearing male child at 5 weeks of life just before HSCT. Superimposed on the picture is a graphic illustration of the early life of hypothetical children with SCID, some without TREC analysis, definitive diagnosis, and treatment with decreasing quality of life, opportunistic infections, failure to thrive, wasting, and death, and others with early diagnosis predicted by means of TREC analysis and molecular DNA assays who engraft and survive with an excellent quality of life and healthy future (Fig 1).

Hopefully, this issue of the *Journal*, with its thematic emphasis on early diagnosis and treatment of infants with SCID, will add to the groundswell of patients and politicians to find the sufficient resources to save these precious children with congenital immune deficiency through implementation of the relatively and inexpensive TREC test that will make the difference between death and life and save collective society more than \$1 million per undiagnosed and untreated child.

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