Newborn Screening

Chapter 26

Severe Combined Immune Deficiency (SCID) leads to life-threatening infections unless the immune system can be restored through a bone marrow transplant, enzyme replacement or gene therapy. Infants with SCID who lack a family history have been diagnosed in the past only after developing serious infections. Early identification of SCID through screening of all newborns can make possible life-saving intervention before infections occur. Currently, several states have adopted the T-cell receptor excision circle (TREC) assay as part of their routine newborn screening programs. TREC screening has identified infants with most forms of SCID and also some infants with very low T-lymphocytes due to other conditions.

Why Screen for SCID?

The absence of T-cell and antibody immunity causes severe infections, diarrhea and failure to thrive. These are the problems that bring infants with SCID to medical attention. The infections experienced by the child with SCID are often caused by weakly pathogenic, opportunistic organisms, organisms that which would not make a child with an intact immune system ill. Prior to 1968, when the first successful bone marrow transplant was performed, SCID was always fatal. Now it can be treated by transplantation of bone marrow stem cells from a healthy donor, or by enzyme replacement or even gene therapy.

Knowing how SCID is inherited has permitted some families, often following tragic loss of an affected infant due to infection, to make the diagnosis in subsequent affected children at birth, or even before birth. In these circumstances, early treatment of infants with SCID who have avoided infections has led to a very high likelihood of survival free of complications. Population based newborn screening for SCID is based on the recognition that pre-symptomatic identification and treatment would improve survival for all infants born with SCID, not just those with a known affected relative.

Screening Test for SCID: T-cell Receptor Excision Circles (TREC)

Population based newborn screening is different from testing undertaken by immunologists confronted with a known or suspected case of immunodeficiency in their practice. Screening tests are performed on a large scale in centralized state public health laboratories that use blood from a heel stick that is spotted onto filter paper and dried, as first developed in 1963 by Robert Guthrie for population based testing of newborns for phenylketonuria. Dried blood spots (DBS) can be handled by automated testing and tracking methods, enabling state laboratories to run thousands of samples at a time. The typical newborn screening test is done on a 1/8" disc that is punched out of the DBS.

(Screening Test for SCID continued)

Unlike individual clinical tests done because of suspicion for a disease by either genetic or clinical information, a screening test looks for a rare, but serious condition in all infants, the vast majority of whom will not have the condition. Therefore, false negative results, or the failure to identify true cases, must be kept to an absolute minimum. On the other hand, false positive results produce anxiety and make follow up testing necessary that also needs to be minimized.

The first suggestion that all newborns be screened for SCID grew from recognizing that the majority of cases could be identified by a complete blood count and differential to determine the absolute number of lymphocytes. T-cells are approximately 70% of lymphocytes in healthy infants, and absence of T-cells causes the total lymphocyte count of most infants with SCID to be low. However, some forms of SCID are associated with the presence of B-lymphocytes, and maternal T-cells are also sometimes found in the blood of infants with SCID. Therefore lymphocyte counts, though simple to perform but require a venipuncture, would not capture all SCID cases. Moreover, T-cell counts cannot be measured in DBS.

Therefore, the TREC test was developed. TREC are circular DNA molecules formed within T-cells developing in the thymus. TREC DNA circles are measured by a technique called polymerase chain reaction (PCR). Normal infant blood samples have one TREC per 10 T-cells, reflecting the high rate of new T-cell generation early in life. Infants with SCID lack TREC altogether.

Occasionally, DBS fail to show TREC DNA for technical reasons; such samples need to have repeat determinations, sometimes requiring a new blood spot

Conditions Found by Screening for Low or Absent TREC

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Typical SCID, due to defects that include IL2RG (X-linked), *ADA, IL7R, JAK3, RAG1, RAG2, DCLRE1C (Artemis), TCRD, TCRE, TCRZ,* and *CD45*
Leaky SCID or Omenn syndrome, due to mutations in typical SCID genes that do not completely abolish gene function
Variant SCID, with persistently low T-cells but no defect in a known SCID gene
Syndromes with variably affected cellular immunity that may be severe, including: • Complete DiGeorge syndrome or partial DiGeorge syndrome with low T-cells • CHARGE syndrome • Jacobsen syndrome • Trisomy 21 • RAC2 dominant interfering mutation • DOCK8 deficient hyper-IgE syndrome • Cartilage hair hypoplasia
Low T-cells as a consequence of other conditions, including: • Neonatal cardiac surgery • Neonatal leukemia • Gastrointestinal malformations • Extreme prematurity (resolves to normal with time) • Intrauterine growth retardation

(Screening Test for SCID continued)

from the infant. Repeated unsatisfactory tests where PCR fails as well as tests indicating low or absent TRECs need to be followed up with a liquid blood sample from the infant that is tested for total lymphocyte numbers and subsets of T, B and natural killer (NK) cells as well as naïve and memory T-cells by flow cytometry. Infants with abnormally low numbers of T-cells should be seen promptly by a pediatric immunologist to determine whether the infant has SCID.

In addition to SCID, other conditions in which T-cell numbers are low can be flagged by TREC testing, as listed in *Table 1*.

The TREC method was first adapted to statewide testing in Wisconsin, followed by Massachusetts, California and New York. Now many other states are conducting TREC screening for SCID, and still more are in the planning stages of offering this testing. These programs have successfully identified SCID and related conditions, allowing infants to receive prompt treatment without the burden of devastating infections. In California alone, at time of publication, 11 cases of SCID, three cases of leaky SCID and Omenn syndrome, four cases of variant SCID, and 15 cases of low T-cells in association with other syndromes have been found. To see if your state screens for SCID, visit www.primaryimmune.org.

TREC Testing - Good, but Not Perfect

TREC newborn screening followed by lymphocyte subset measurement has now been proven to have clinical utility in several states. Many infants with otherwise unsuspected SCID or related T-cell disorders have been referred for prompt evaluation and treatment, and reports of successful outcomes are emerging. As more experience accumulates and more states add newborn TREC screening, it will be important to document outcomes of the current programs. Not only the total incidence but also the severity spectrum and relative incidence of these rare conditions in different population subgroups remain to be defined.

The Newborn Screening Translational Research Network (NBSTRN) has established a program for tracking and reporting cases found by prospective screening so that diagnoses and screening test performance can be compared and analyzed between states and in the country as a whole.

Not all T-cell deficiency diseases are detected by the TREC test. Diseases in which T-cells develop in the thymus to the point of production of the DNA circles but have impaired function are missed. For example, newborns with Zap70 deficiency, MHC Class II deficiency and NF-kappa-b essential modulator (NEMO) deficiency have had normal TRECs, as has one patient with late-onset adenosine deaminase (ADA) deficiency.

Future Issues for Newborn Screening for Immune Disorders

Now that TREC screening has become available and its effectiveness has been shown, spreading its implementation to all states is important. As screening becomes widespread, the true incidence and proportions of each type of SCID can be determined.

Immediate measures can be put in place for all patients—immunoglobulin replacement therapy and prophylactic antibiotics, avoidance of live vaccines, protection from exposure to infections—while the best type of definitive treatment is planned. Optimal transplant protocols for very young infants with SCID remain controversial, but with newborn screening these protocols can be established by multicenter studies.

All primary immunodeficiency diseases, not just SCID, stand to benefit from early diagnosis. Continued advances in molecular and genomic technology may soon allow screening for lack of B-cells, with testing for B-cell kappa chain excision circles, or KRECs.

Moreover, it is possible that future newborns will have extensive testing for DNA mutations or sequencing of their entire genome, from which a blueprint of risks for a great variety of conditions affecting health can be ascertained. Even predisposition to the more common multifactorial immune disorders with later onset may become possible through deep sequence analysis of DNA from newborns. However, since the mere presence of a mutation does not fully predict phenotype for these conditions, much more needs to be learned about the true predictive value of each proposed type of screening.