Chapter 26
Newborn Screening

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Newborn Screening for Severe Combined Immunodeficiency

What Is SCID?

Severe Combined Immunodeficiency (SCID), pronounced "skid," includes some of the most severe forms of primary immunodeficiency diseases (PI), and is characterized by an absence of T cells and little or no B cell lymphocyte function (NK lymphocytes are also affected in some cases), resulting in life-threatening infections that lead to death unless the immune system is restored through treatment. Treatment is usually done by either hematopoietic stem cell transplantation (HSCT), also known as bone marrow transplantation, or, in certain types of SCID, through enzyme replacement therapy and/or gene therapy. SCID is caused by genetic (inherited) defects. Most babies born with SCID appear well at birth. Prior to the development of a newborn screening (NBS) test in 2008, these babies were diagnosed only after they developed serious infections. Now, it is possible to diagnose and treat infants before the development of life-threatening infections.

What is the screening test for SCID?

All newborn babies in the U.S. have blood from a heel stick spotted onto a filter paper and dried. Using a sample from this Newborn Dried Blood Spot (DBS), states in the U.S. perform over 30 tests for conditions such as phenylketonuria and congenital hypothyroidism. These tests are performed routinely on all newborns. Usually the blood spot is collected at the birth hospital (or at home when there is a home birth), and then they are sent to the state Newborn Screening laboratory. Each state performs these tests on a few hundred to thousands of infants each week. Abnormal results require follow-up with additional tests to confirm the diagnosis and a visit to their doctor or a specialist.

While originally intended to screen infants for common conditions, newborn screening tests are also performed for rare conditions that result in significant disease or carry a high risk of death early in life. Early diagnosis of these conditions is important so that early treatment can be started to reduce some of the bad outcomes, and in some cases treat the disease. As of December 2018, every state in the U.S., as well as Washington, DC and Puerto Rico, undertakes NBS for SCID for every child born, measuring a circular piece of DNA, the T cell receptor excision circle (TREC), using a small (1/8") disc punched out of the DBS. This test has been effective in identifying infants with most forms of SCID, as well as infants with other conditions causing very low T cells.

Why Screen for SCID at Birth?

Since SCID is inherited, prior to the use of NBS, only families that previously had a child with known or suspected SCID had the opportunity to identify SCID at birth in subsequent children, before the onset of symptoms. In these families, the infants diagnosed soon after birth had a better chance of survival with fewer complications. This demonstrated the importance of early recognition, or pre-symptomatic identification of SCID, and early initiation of treatment. Not only was this linked to higher survival rates but also to less hospitalizations and lower healthcare costs. Based on this evidence and information from pilot screening in Wisconsin, population-based newborn screening was recommended for SCID. After this recommendation was published in 2010 and followed by years of advocacy by organizations including the Immune Deficiency Foundation (IDF), each year more states began implementing NBS for SCID. As mentioned earlier, as of December 2018, every state in the U.S., as well as Washington, DC and Puerto Rico, screens for SCID.

How Does the TREC Test Identify Infants with SCID?

The TREC test uses a polymerase chain reaction (PCR) to measure the amount of TREC DNA molecules from the DBS. The number of copies of TREC DNA correlates very well to the number of new T cells coming out of the thymus gland after they go through the proper development. Healthy newborn babies usually have a large number of T cells in their blood and approximately one copy of TREC per 10 T cells. The number of TREC in the blood drops very rapidly with age,

and adults typically have very low numbers of TREC in their blood. The absence (or near absence of) T cells and TREC is a very reliable way of identifying an infant who has SCID. Occasionally, some of the mother's T cells can be found in the baby's blood, so measurement of T cell numbers alone might not diagnose SCID. Since the mother's T cells will have a low TREC number, the newborn screening test would still be abnormal and signal the presence of possible SCID. It is important to remember, however, that the TREC test does not always diagnose SCID. We will discuss some of the other conditions that can also cause an abnormal TREC test. That is why secondary or confirmatory testing is critical.

What other conditions cause low or absent TREC at birth?

Low T cells at birth (causing the low/absent TREC) can be caused by other conditions causing a low production of T cells, including:

- Extreme prematurity (mainly infants born before 30 weeks)
- DiGeorge (22q11.2 deletion) syndrome
- Jacobsen syndrome
- Trisomy 21 (Down syndrome)
- CHARGE syndrome
- A defect in FOXN1 causing poor thymus development
- DOCK8 deficient Hyper-IgE syndrome
- Ataxia-Telangiectasia
- Other forms of combined immunodeficiency

Additionally, the TREC may be low due to losses of T cells in the intestines (intestinal lymphangiectasia, other gastrointestinal malformations) in babies with congenital cardiac disease or who have had cardiac surgery, or in babies with severe hydrops (generalized swelling). Rarely low TREC can be caused by destruction of T cells as seen in neonatal leukemia and some forms of acute HIV infection. Lastly, there is a group of infants with an abnormal TREC newborn screen who have low CD4 T cells for unknown reasons. These infants have been described as having idiopathic T cell lymphopenia.

What Is a "False Positive" Test?

The term false positive means that an abnormal test result happens in an unaffected person. Most of the time this occurs due to an error in the testing. The TREC test also measures the number of copies of another unrelated control gene to check the performance of the test in case the PCR fails to detect any TREC. This can occur for a variety of technical reasons from the way the blood spot was collected to problems performing the test in the laboratory. Therefore, if both TREC and the control gene fail to amplify, the PCR test is deemed unsatisfactory, and the state newborn screening laboratory will ask for a new blood spot to be collected.

What Happens Next for Infants with Low or Absent TREC?

If the TREC DNA is very low but the control gene DNA level is satisfactory, a liquid blood sample, collected through a venipuncture, will be tested to measure the total number of lymphocytes, and the numbers of T, B and natural killer (NK) cells by flow cytometry (a highly technical laser instrument that can detect and measure single cells labelled with a fluorescent dye). It is recommended that the proportion or number of naïve and memory T cells by flow cytometry also be measured to identify whether these T cells could come from the mother because T cells from the baby should be mostly naïve T cells. The blood sample for T cell measurement might be collected by the pediatrician, but the results are usually viewed by specialists working with the newborn screening program. All infants with abnormally low numbers of T cells should be seen as soon as possible by a pediatric immunologist or a specialist with experience diagnosing and caring for infants with SCID and other types of PI.

The immunologist will usually order additional tests to diagnose the cause for the low T cell numbers. These tests can include: measures of T cell function, determining whether any T cells seen in the baby's blood are from the mother, and genetic tests to identify the genetic cause of SCID or to identify any other suspected syndrome.

How Well Does TREC Testing Identify Infants with SCID?

TREC based NBS has been shown to be a good test for identifying infants who may have SCID. Over 10 states in the U.S. have now published their cumulative experience over several years showing that infants with abnormal TREC at birth are referred to treatment centers and can undergo treatment much earlier than previously. Information is still being collected on an ongoing basis. As more states publish data on their implementation of SCID NBS, we will have more information on the outcomes of these programs. The Newborn Screening Translational Research Network (NBSTRN) collects information nationally on diagnoses and screening test performance. At this time, there are no reports of infants with typical SCID (for example, those with absent T cells) who were missed due to a TREC test. There are rare forms of T cell defects, however, where some T cells develop but have poor function, such as atypical forms of SCID due to ZAP 70 deficiency, MHC Class II deficiency, or combined immune defects due to NF kappa-B essential modulator (NEMO) deficiency. In addition, one individual with late-onset adenosine deaminase (ADA) deficiency (ADA-SCID) had a normal TREC early on.

What Steps Occur If SCID Is Suspected?

If SCID or another significant T cell deficiency is suspected, it is a priority to take immediate steps to protect the infant from developing any infections. Infants should not attend daycare, and exposure to persons with possible infections should be avoided. Starting immunoglobulin (Ig) replacement therapy, prophylactic antibiotics, and antiviral and antifungal medications is important. Live vaccines, such as the rotavirus (oral) vaccine, should be avoided. If the infant requires a blood transfusion, the doctors and hospital should use blood that is depleted of white blood cells (or irradiated to destroy any viable lymphocytes) and does not contain any cytomegalovirus (CMV). Mothers are not advised to breast feed their infants. The risk of transmission of CMV through the breast milk is significant in any mother who has previously had an infection with CMV. This risk persists for years after exposure to CMV. Many mothers are tested for the CMV antibody during their pregnancy; this does not guarantee, however, that they are not exposed to CMV later in pregnancy or after delivery. Commercial infant formula or pasteurized breast milk are safe alternatives.

What Is Next for Newborn Screening for Other Types of PI?

The use of PCR testing for other types of PI has been considered, such as Kappa rearrangement excision circles (KRECs) to estimate immature B cell numbers that are low both in some forms of SCID and in those with a lack of immunoglobulin due to the absence of B cells, such as in X-linked Agammaglobulinemia (XLA). In the future it may be possible to sequence the entire genome and identify a myriad of diseases or predispositions to common multifactorial immune disorders. This testing is still in the research stage and will need to be fully studied to better understand the relationship between a gene mutation and the development of a disease to ensure that only disease causing mutations will be identified.

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