The Wisconsin approach to newborn screening for severe combined immunodeficiency

James Verbsky, MD, PhD,[a,b] Monica Thakar, MD,[b,c] and John Routes, MD[b] *Milwaukee, Wis*

Severe combined immunodeficiency (SCID) is a life-threatening disease of infants that is curable with hematopoietic cell transplantation if detected early. Population-based screening for SCID using the T-cell receptor excision circle (TREC) assay began in Wisconsin in 2008; 5 infants with SCID or other forms of severe T-cell lymphopenia (TCL) have been detected, and no infants with SCID have been missed. This review will provide an overview of the TREC screening assay and an update of the findings from Wisconsin on all infants screened from January 1, 2008, until December 31, 2010. Importantly, we give practical recommendations regarding newborn population-based screening using the TREC assay, including the evaluation and care of infants detected. (J Allergy Clin Immunol 2012;129:622-7.)

Key words: *Severe combined immunodeficiency, T-cell receptor excision circle, lymphopenia, newborn screening*

Abbreviations used

AGA: Adjusted gestational age
HCT: Hematopoietic cell transplantation
NBS: Newborn screening
NK: Natural killer
RAC2: RAS-related C3 botulinum toxin substrate 2 RNaseP
RT-qPCR: Real-time quantitative PCR
SCID: Severe combined immunodeficiency
sTCL: Severe T-cell lymphopenia
TCL: T-cell lymphopenia
TREC: T-cell receptor excision circle

Severe combined immunodeficiency (SCID) is a life-threatening disease of infants caused by a heterogeneous group of genetic defects that prevent the normal development of T cells.[1] Regardless of the genetic cause, all infants with SCID exhibit a severe deficiency of naive T cells with variable decreases in B and natural killer (NK) cell counts. Infants with SCID are typically well at birth. However, once the levels of maternally derived antibodies wane, they become susceptible to a variety of bacterial, viral, and opportunistic infections and frequently exhibit marked failure to thrive. SCID is a pediatric emergency and is uniformly fatal without hematopoietic cell transplantation (HCT). The diagnosis and treatment of SCID in the first 3.5 months of life, before the development of life-threatening infections, markedly improves outcomes.[2,3]

The diagnosis of SCID is frequently delayed or missed altogether, in part because of the normal physical appearance of these infants in the majority of cases. Consequently, clinical immunologists have long advocated for the inclusion of SCID in newborn screening (NBS). In 1968, Wilson and Jungner[4] proposed objective criteria for inclusion of a disease for population-based NBS. These criteria have undergone some modifications but are widely accepted, and some of the salient criteria are summarized in Table I. Although SCID fulfills all of these criteria, a major impediment in implementing NBS for SCID was the lack of an economic, sensitive, and specific screening test that was amenable for NBS. Such a screening test would detect all cases of low or absent naive T cells and, if possible, use NBS cards currently in use. The development of the T-cell receptor excision circle (TREC) assay for use in NBS for SCID and its subsequent optimization for population-based screening overcame this major obstacle.[5,6] The TREC assay enumerates the number of TRECs by using real-time quantitative PCR (RT-qPCR) on DNA extracted from NBS cards. TRECs are nonreplicative pieces of DNA formed during T-cell receptor gene rearrangement during development in the thymus. The δRec-ψJα TREC is produced on T-cell receptor rearrangement in approximately 70% of all T cells that express the αβ T-cell receptor.[7] Quantification of TRECs by means of RT-qPCR with DNA isolated from NBS blood spots is a surrogate marker for the number of circulating naive T cells that have recently emigrated from the thymus.[8]

In January 2008, funded by matching contributions from the Jeffrey Modell Foundation and the Children's Hospital of Wisconsin, as well as financial support from the Wisconsin Laboratory of Hygiene, Wisconsin became the first state to screen all infants for SCID by using the TREC assay.[9] Results from the first year of screening in Wisconsin demonstrated that the TREC assay detects infants with profound T-cell lymphopenia (TCL) and led to the diagnosis and successful HCT of an infant with RAS-related C3 botulinum toxin substrate 2 RNaseP (*RAC2*) mutation.[10] With proof that the TREC assay could detect TCL in

From the Divisions of [a]Rheumatology, [b]Hematology/Oncology/Transplant, and [c]Allergy/Clinical Immunology, Department of Pediatrics, Children's Research Institute, Medical College of Wisconsin.

Supported by the Children's Hospital of Wisconsin, the Jeffrey Modell Foundation, the Wisconsin State Laboratory of Hygiene, and the Centers for Disease Control and Prevention (U01 EH000365).

Disclosure of potential conflict of interest: J. Verbsky has received honoraria from Baxter, Illumina, and the Jeffrey Modell Foundation. M. Thakar declares that she has no relevant conflicts of interest. J. Routes has received research support from the National Institutes of Health.

Received for publication October 14, 2011; revised December 6, 2011; accepted for publication December 9, 2011.

Available online January 11, 2012.

Corresponding author: John Routes, MD, Pediatrics, Children's Hospital of Wisconsin, Medical College of Wisconsin, 9000 W Wisconsin Ave, Milwaukee, WI 53226. E-mail: jroutes@mcw.edu.

0091-6749/$36.00

doi:10.1016/j.jaci.2011.12.004

TABLE I. Summary of Wilson and Jungner criteria for NBS

Criteria for population-based NBS
Prevalence of disease warrants cost of NBS
Disorder not readily identified by means of physical examination
Disease must cause serious medical complications
Early diagnosis and treatment of disease improves prognosis
Acceptable, sensitive, specific, economic, and proved screening test must be available

conjunction with compelling evidence that SCID met the conventional criteria for NBS, the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children unanimously agreed that the TREC assay should be added to the uniform panel of tests recommended by the US government for NBS of infants in all states. This recommendation was approved by the Secretary of Health and Human Services, Kathleen Sibelius, in 2010.

RESULTS OF 3 YEARS OF NBS IN WISCONSIN

During the first 3 years of screening in Wisconsin, 207,696 infants were screened with the TREC assay.[11] In total, 72 full-term infants (0.035%) had an abnormal TREC assay result (low TREC counts and normal β-actin levels) during the first 3 years of testing, which included 63 infants found to have abnormal results on initial testing and 9 infants reclassified as having abnormal results after repeat testing because of prematurity or low β-actin levels. Of the abnormal results, 38 infants had normal T-cell counts, as determined by means of flow cytometry, resulting in a false-positive rate of 0.018% and specificity of 99.98%. Thirty-three infants were found to have TCL of varying degrees, and 1 family refused further testing. Thus the positive predictive value of the test predicting TCL of any cause was 45.83%. Of the 33 infants with TCL, 19 (58%) had secondary causes for TCL, such as anatomic abnormalities of the lymphatics, chromosomal abnormalities, multiple congenital anomalies, or presumed metabolic disorders.[11] In all of these cases, TREC counts were detectable but low. Of the remaining 14 infants, there were 5 with reversible TCL, 4 with 22q11.2 deletion syndrome, and 5 with SCID/severe T-cell lymphopenia (sTCL).

The laboratory characteristics of the 5 infants with SCID/sTCL detected by using the TREC assay are summarized in Table II. The first infant identified by means of NBS for SCID/sTCL who required HCT presented with multiple abscesses and was found to have a dominant negative mutation in the *RAC2* gene, resulting in defects in both neutrophil and T-lymphocyte numbers.[10] One infant with SCID had adenosine deaminase deficiency. One infant with SCID exhibited deficiencies of T and B cells with normal NK cell numbers, although sequencing for mutations in recombination-activating gene *(RAG)* 1 and 2 or the DNA cross-link repair 1C gene *(DCLRE1C)* were negative. A fourth infant was identified with sTCL but with relatively normal B-cell and NK cell counts. Identification of this infant led to the evaluation of an older sibling with a similar phenotype who had recurrent opportunistic infections and autoimmune enteropathy.[9] The sibling subsequently underwent HCT, and the proband is awaiting transplantation. Analysis of the fifth infant similarly demonstrated sTCL with relatively normal NK and B cell counts. Sequence analysis for mutations in *CD3*, ζ chain–associated protein 70 KD *(ZAP70)*, and IL-7 receptor *(IL7R)* or microdeletions at 22q11.2 failed to detect a known defect. The infant's T cells demonstrated virtually no proliferative response to IL-7; the patient has persistent profound TCL (naive CD3 T-cell counts, 200-300/μL) and undetectable TRECs, and therefore this patient underwent matched unrelated HCT.[11] In total, 3 infants and 1 sibling given diagnoses of SCID/sTCL based on results of the TREC assay have undergone HCT, and all are engrafted and doing well to date. In the cohort of infants screened for SCID/TCL to date in Wisconsin, there have been no reported cases of SCID that have gone undiagnosed by means of NBS.

Finally, the sensitivity and specificity of a result of zero TRECs with a normal β-actin level for SCID/sTCL cannot be overstated. In all cases of SCID/sTCL, an initial TREC count of zero was obtained, and the values remained very low during the first few months of testing. Thus the absence of detectable TRECs is highly predictive of sTCL and requires an urgent clinical evaluation, including anticipatory guidance for the infant's parents and physician (see below).

THE TREC ASSAY IN PREMATURE INFANTS

Early in the implementation of the NBS program for SCID, it became apparent that the numbers of abnormal (ie, low TREC counts and normal β-actin levels) or inconclusive (ie, low TREC counts and low β-actin levels) TREC assay results were higher in premature infants (adjusted gestational age [AGA] ≤ 37 weeks) compared with those seen in full-term infants.[9,11] In 3 years of screening, 63 (0.03%) full-term infants were classified as having abnormal results, and 51 (0.025%) had inconclusive results on initial testing, whereas 94 premature infants were classified as having abnormal results (0.045%), and 241 (0.116%) were classified as having inconclusive results. Due in part to the high false-positive rate of an abnormal TREC assay result in premature infants,[9,11,12] it is the standard operating procedure in Wisconsin to repeat the TREC assay in premature infants until they reach an AGA of 37 weeks or greater before performing lymphocyte subset analysis by means of flow cytometry (Fig 1). There is considerable variation from state to state on the standard operating procedures for retesting premature infants (lymphocyte subsets vs a repeat TREC assay). In highly populated states, such as California or New York, logistical difficulties might legitimately preclude retesting premature infants with the TREC assay. However, we believe the retesting procedure in Wisconsin can lead to a significant reduction in the overall expense of the program because the estimated cost of a repeat TREC assay is between $5 and $6, whereas standard lymphocyte enumeration panels cost more than $400.

There are several factors that might contribute to the increased rate of false-positive TREC assay results in preterm infants, including the lack of studies that define normal TREC counts as a function of gestational age and the administration of maternal corticosteroids,[13] which might reduce peripheral T-cell counts. The increased rate of inconclusive results in premature infants is in part due to the use of blood that was obtained from indwelling catheters that can dilute the sample or contain substances, such as heparin, that inhibit the RT-qPCR assay.[14]

There were instances in which infants with either abnormal or inconclusive TREC results died before obtaining a lymphocyte subset test performed by means of flow cytometry. However, a detailed retrospective chart review of all such infants who died in the first 2 years of NBS in Wisconsin demonstrated that the vast majority of infants (33/39 [85%] infants) were extremely