

The Wisconsin approach to newborn screening for severe combined immunodeficiency

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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;■■■:■■■-■■■.)

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

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}

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

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 Jungner⁴ 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 δ Rec- ψ J α TREC is produced on T-cell receptor rearrangement in approximately 70% of all T cells that express the $\alpha\beta$ 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

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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/ μ L) 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

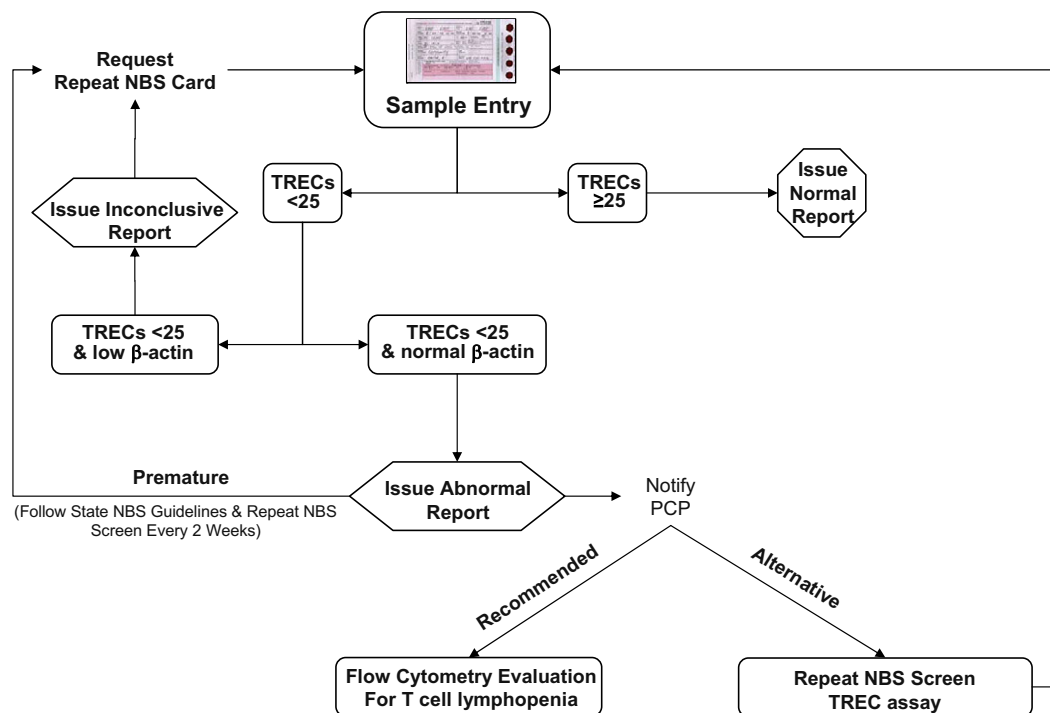
There were instances in which infants with either abnormal or inconclusive TREC results died before obtaining a lymphocyte subset test performed by means of flow cytometry. However, a detailed retrospective chart review of all such infants who died in the first 2 years of NBS in Wisconsin demonstrated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

TABLE II. Laboratory and clinical summary of infants detected with SCID/sTCL

Case no.	TREC	CD3 (naive)	B cell	NK cell	Mito	Treatment/outcome
1. <i>RAC2</i>	0,4,0	2869 (2353)	166	291	87%	5/6 UCBT
2. <i>ADA</i>	0,0,0	0 (0)	0	0	ND	On enzyme replacement, gene therapy pending
3. T ⁺ B ⁻ NK ⁺	0,0,0	70 (49)	42	752	3%	8/8 UCBT
4. T ⁺ B ⁺ NK ⁺	0,4,3	411 (201)	1342	346	19%	Sister with similar defect, transplantation pending
5. T ⁺ B ⁺ NK ⁺	0,0,0	406 (280)	263	956	47%	IL-7 signaling defect, 10/10 MUD

All cell counts are in cells per microliter of blood.

ADA, Adenosine deaminase; *Mito*, T-cell mitogen response as a percentage of control value; *MUD*, HLA-matched unrelated donor; *ND*, not done; *TREC*, TREC count/ μ L of blood on initial testing; *UCBT*, umbilical cord blood transplantation.

**FIG 1.** Screening algorithm. *PCP*, Primary care provider.

premature (AGA < 33 weeks) and died from complications related to prematurity, congenital abnormalities, or complications of pregnancy. There was no evidence that SCID contributed to the cause of death in these neonates.¹²

Finally, there is also considerable state-to-state variability in the technique used to validate the presence and integrity of DNA in each dried blood spot sample. Massachusetts, Texas, and New York link the amplification of TRECs to simultaneous amplification of the internal reference gene ribonuclease P (*RNAseP*). In contrast, screening laboratories in Wisconsin, California, and Pennsylvania amplify a control gene only after an abnormal initial result is reported. In Wisconsin more than 98% of initial TREC assays did not require repeat testing with concomitant β -actin amplification.¹¹ Because the reagents needed for β -actin amplification are infrequently used, this strategy should reduce the overall expense of the screening program.

PITFALLS AND DILEMMAS

It is generally accepted that a diagnosis of SCID can be made with a T-cell count of less than 200 cells/ μ L and particularly

with mitogenic responses of less than 10% of control values, even in the absence of a molecular cause. This definition, however, would have only applied to 2 infants detected in Wisconsin, highlighting that the TREC assay detects known and unknown causes of SCID/TCL. The infant with a *de novo* mutation in the *RAC2* gene had relatively normal T-cell counts and T-cell proliferative responses but had zero TRECs on initial testing and had a serious immune defect affecting neutrophils and T cells.¹⁰ When infants are born with absent TRECs but with greater than 200/mm³ naive T cells and no known molecular defect, it becomes an even more difficult diagnostic and ethical dilemma to confidently state that the infant has a form of TCL that requires HCT. In our cohort of 5 infants with sTCL, 2 infants had greater than 200/mm³ T cells and no genetic defect (Table II). With the institution of prophylactic antibiotics and intravenous immunoglobulin, SCID-defining opportunistic infections did not occur. T-cell proliferative responses were reduced but not to less than 10% of normal values, demonstrating that these studies do not rule out a serious immune defect. For example, the sibling of infant case 4 (Table II) had greater than 200/mm³ naive T cells, T-cell proliferative responses of greater

TABLE III. Recommended supportive care anticipatory guidelines for infants with suspected SCID

Guideline	Reason
Avoid public places, day care	Prevent transmission of community-acquired diseases
Limited contact with young children	
Strict hand washing	
No breast-feeding	Prevent transmission of CMV
Boil ingestible water	Prevent cryptosporidium infection
Avoid all live and live attenuated vaccines (MMR, varicella, rotavirus, FluMist*)	Prevent infection with vaccine-related viruses
Blood product leukodepletion and irradiation essential; CMV negative when available	Prevent transmission of CMV and graft-versus-host disease

CMV, Cytomegalovirus; MMR, measles, mumps, rubella.

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than 10%, and relatively normal immunoglobulin levels and vaccine titers but clinically presented phenotypically as having an SCID variant with failure to thrive and recurrent and opportunistic infections.⁹

These situations highlight the importance for patients identified by means of NBS for SCID/sTCL to undergo a comprehensive immunologic evaluation by a trained clinical immunologist. In cases in which the diagnosis of SCID/TCL is unclear, we have taken a cautious approach, starting these children on immunoglobulin replacement, antimicrobial prophylaxis, and appropriate environmental controls while further investigations were pursued. These infants are closely monitored in hopes that if T-cell recovery will occur, it will do so before the development of infectious sequelae. Maternal engraftment is excluded by variable number tandem repeat testing or HLA typing, T-cell mitogen studies are performed and repeated, and naive T-cell counts and TREC counts are enumerated over time. Consultation with experts from around the country and advanced immunologic testing is pursued in these cases. Novel approaches, such as whole-genome/exome sequencing, to define the genetic cause of the TCL in these patients are currently being used in Wisconsin and elsewhere.¹⁵

ANTICIPATORY GUIDANCE OF INFANTS DETECTED WITH SCID/TCL

With the inception of NBS for SCID, newborns with SCID/sTCL are being identified within the first few weeks of life, before the onset of illness or infections. We have developed a multidisciplinary program, with input from both the clinical immunology and bone marrow transplantation services, to manage infants with high suspicion for SCID/sTCL. Our primary goal is to prevent infections that could predispose infants to end-organ damage and transplant-related mortality. We have also developed supportive care and infectious disease prophylaxis guidelines based in part on national consensus guidelines, including isolation parameters and pharmacologic therapy, which are started at the time of initial contact with the patient and continued through the immunologic diagnostic workup until HCT.¹⁶

When SCID is suspected in an infant, HLA typing is sent on the patient and immediate family members. The transplant service initiates an unrelated donor search when a suitable donor with an HLA match is not found within the family. While the immunologic workup of infants detected is underway, frequent meetings between the clinical immunology service and the bone marrow transplantation service occur. Once a diagnosis of SCID/sTCL is confirmed, care is formally transferred to bone marrow transplantation service.

WHERE SHOULD PATIENTS BE MANAGED? THE DECISION OF INPATIENT VERSUS OUTPATIENT CARE

Infants suspected of SCID but without infectious symptoms are cared for at home, and we use several guidelines to prevent infection (Table III). We also require that the infant live within 1 hour's drive of our hospital or another appropriate hospital well versed in taking care of sick children from which transfer to our hospital would occur. Infants whose social surroundings, home surroundings, or both are worrisome for poor care, follow-up, or family's lack of understanding of the prophylaxis measures might also require inpatient admission to care for the child.

A high degree of suspicion for infections is critical because infections can be clinically asymptomatic. If a newborn has any concern for infection, including fever without obvious source, admission to the inpatient unit is mandatory. These patients are placed in positive-pressure rooms on the bone marrow transplantation unit with mandatory hand washing and limited visitation, including no sick contacts.

PROPHYLAXIS OF INFECTIOUS DISEASE AND OTHER MANAGEMENT ISSUES

The infectious diseases department is consulted, and all newborns with possible SCID undergo a panel of PCR tests to evaluate for viral disease (Table IV). If results of these PCR assays are negative, surveillance PCR assays are repeated monthly or bi-monthly during the pretransplantation period. The frequency of PCR surveillance increases based on clinical suspicion of new infection (eg, fever or irritability) or if the initial PCR result is positive. Other laboratory studies (eg, lumbar puncture or blood cultures) are performed as clinically indicated. Infants with positive test results for viral infection are hospitalized, and antiviral therapy is implemented. Serologic assays, except for HIV-1 infection, are not obtained because specific antibody production is absent in infants with SCID and variably present in infants with TCL. Infants are screened for antibodies to HIV-1, which would indicate maternal HIV infection and would require immediate treatment. Additional mandatory testing, as required by the National Marrow Donor Program, along with imaging studies to evaluate for infection, are performed during the pre-HCT workup evaluation (Table IV).

Infants identified as having possible SCID or with sTCL undergo a prophylaxis regimen designed to prevent bacterial, fungal, and viral infections (Tables III and V). Infants with SCID or sTCL do not receive any live vaccinations, and we

TABLE IV. Recommended infectious disease screening at initial NBS referral and before BMT

Test	At referral for NBS	Monitoring during pre-HSCT period	During pretransplantation evaluation (1-2 wk before HSCT)
Blood PCR tests			
CMV, EBV, adenovirus, HHV6	X	Every 2-4 wk if negative result	X
Hepatitis B, hepatitis C, HSV1/2, HIV-1	X		X (omit if performed <30 d before HSCT and negative result)
NMDP required before HSCT: WNV			X
Blood antibody testing			
HIV-1	X		X
NMDP required before HSCT: HIV-2, HTLV1/2, HepBsAg, Hep B core, hepatitis C, CMV, <i>T cruzi</i> , STS			X
Nasopharyngeal swabs for viral PCR and viral culture (regardless of symptoms)			
Influenza A and B; parainfluenza 1, 2, and 3; RSV; adenovirus; and HMPV	X		X
Imaging evaluation			
Chest radiography; CT scans of head, neck, chest, abdomen, and pelvis; urinalysis	Only if clinically indicated	Only if clinically indicated	X

BMT, Bone marrow transplantation; *CMV*, cytomegalovirus; *CT*, computed tomography; *HepBsAg*, Hepatitis B surface antigen; *HepB core*, Hepatitis B core antibody; *HHV6*, human herpes virus 6; *HMPV*, human metapneumovirus; *HSCT*, hematopoietic stem cell transplantation; *HSV1/2*, herpes simplex virus 1/2; *HTLV1/2*, human T-lymphotropic virus 1/2; *NMDP*, National Marrow Donor Program; *RSV*, respiratory syncytial virus; *STS*, serologic test for syphilis; *T cruzi*, *Trypanosoma cruzi* antibody; *WNV*, West Nile virus.

TABLE V. Recommended infectious disease prophylaxis for newborns suspected of having SCID

Prophylaxis	Drug (dose)	Time of initiation	Alternatives	Comments
In newborns				
PCP	Oral TMP-SMX (5 mg TMP/kg once a day for 2 consecutive days weekly)	1 mo old	Oral atovaquone (30 mg/kg once a day)	Verify that bilirubin level is <2 times the upper limit of normal before starting; monitor ALT and AST every 2-4 wk
HSV and VSV	Oral acyclovir (20 mg/kg per dose 3 times a d)	At first visit		
RSV	Palivizumab (15 mg/kg administered intramuscularly)	1 mo old		Given during peak RSV season, typically November-March
General (bacterial/viral)	IVIG (0.4-0.5 g/kg every month)	1 mo old		Monitor troughs monthly and maintain immunoglobulin >600 mg/dL
Fungal	Fluconazole (6 mg/kg once daily)	1 mo old	Micafungin	Follow AST and ALT every 2-4 wk
In family members or close contacts				
Influenza	Inactivated or attenuated influenza vaccine	Seasonally		Attenuated vaccine safe in family members of infant with SCID
Pertussis	Tdap vaccine	Per routine childhood vaccinations		One booster for adolescents (11-12 y old), adults 19-64 y old, and adults >65 years old

ALT, Alanine aminotransferase; *AST*, aspartate aminotransferase; *HSV*, herpes simplex virus; *IVIG*, intravenous immunoglobulin; *PCP*, *Pneumocystis carinii* pneumonia; *RSV*, respiratory syncytial virus; *TMP-SMX*, trimethoprim-sulfamethoxazole; *VSV*, varicella stomatitis virus.

withhold other routine immunizations as well because these children are undergoing intravenous immunoglobulin prophylaxis. We instruct family members and close contacts to be up to date on immunizations against influenza and pertussis. Although transmission of varicella, measles, and rotavirus through vaccination is also highly unlikely,¹⁷ we do not recommend that close contacts or family members be vaccinated unless there is a community outbreak or if there are other special circumstances.

If the infant is hospitalized, a neonatologist or the pediatric hospitalist service is consulted for a general pediatrics evaluation. For outpatients, we maintain continuity with the primary care provider, who can continue monitoring the infant for normal developmental milestones and nutrition. We advise the primary care provider to schedule office visits as the first appointment of the morning to limit waiting room exposures. Finally, the social work service is deeply involved in all cases of inpatient or outpatient management.

CONCLUSION

NBS for SCID/sTCL with the TREC assay is the first step in the early identification and treatment of SCID and other severe immunodeficiencies that present with TCL. Since the initiation of NBS for SCID/sTCL in 2008, there has been considerable progress in implementing nationwide screening. Outcomes to date have been extremely encouraging because all infants given a diagnosis of SCID/sTCL by means of existing NBS have been successfully treated and there have been no missed cases of SCID in the screened population. Questions regarding novel causes of SCID/sTCL and the appropriate course of treatment will continue to arise, and it is our responsibility as participants in the care of these patients to continually reassess our knowledge and practices to ensure the best possible outcomes. A proactive approach to infants detected with SCID/sTCL should be used, including anticipatory guidance, infection surveillance, and broad prophylaxis, to prevent infection until definitive treatment with HCT can be performed.

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