HHS Public Access

Author manuscript

J Allergy Clin Immunol Pract. Author manuscript; available in PMC 2022 May 01.

Published in final edited form as:

J Allergy Clin Immunol Pract. 2021 May ; 9(5): 2060–2067.e2. doi:10.1016/j.jaip.2021.02.006.

Ten Years of Newborn Screening for Severe Combined Immunodeficiency (SCID) in Massachusetts

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Authorship Contributions

AMC, JEH and others in the original SCID NBS workgroup (FAB, BNH, JLS, AMJ, MSP, PEH, HCM, ERC, IS, RBE, AD, LDN, SYP) conceived, began, and optimized the Pilot Program. AMC and SYP planned and supervised the outcomes-data analyses. JEH and CP gathered and curated data, and performed analysis. JEH wrote the original draft of the manuscript and CP, AMC and SYP edited the manuscript. CP, FAB, BNH, JLS, AMJ, MP, PEH, CM, EC, SB, JRF, DF, JEW, NY, LDN, and SYP performed clinical activities related to newborn screening, collected and provided data to the NENSP. All authors reviewed and approved the final manuscript.

Conflict of Interest Statement: All authors attest that they have no conflicts to disclose.

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Abstract

Background: Massachusetts began newborn screening (NBS) for severe combined immunodeficiency (SCID) using measurement of T cell receptor excision circles (TRECs) from dried blood spots.

Objective: We describe developments and outcomes from the first 10 years of this program (Feb 1 2009-Jan 31 2019).

Methods: TREC values, diagnostic, and outcome data from all patients screened for SCID were evaluated.

Results: NBS of 720,038 infants prompted immunologic evaluation of 237 (0.03%). 9/237 were diagnosed with SCID/leaky SCID (4% of referrals *vs* 0.001% general population). Another 7 were diagnosed with other combined immunodeficiencies, and 3 with athymia. SCID/leaky SCID incidence was ~1 in 80,000, while ~1 in 51,000 had severe T cell lymphopenia for which definitive treatment was indicated. All patients with SCID/leaky SCID underwent hematopoietic cell transplant or gene therapy with 100% survival. One patient with athymia underwent successful thymus transplant. No known cases of SCID were missed. Compared to outcomes from the 10 years prior to SCID NBS, survival was higher (9/9 vs 4/7), likely due to a lower rate of infection before treatment.

Conclusions: Our data support a single NBS testing-and-referral algorithm for all gestational ages. Despite lower median TREC values in premature infants, the majority for all ages are well above the TREC cutoff and the algorithm, which selects urgent (undetectable TREC) and repeatedly abnormal TREC, minimizes referral. We also found that low naïve T cell percentage is associated with a higher risk of SCID/CID, demonstrating the utility of memory/naïve T cell phenotyping as part of follow-up flow cytometry.

Keywords

Newborn screening (NBS); SCID; TREC; athymia; prematurity; hematopoietic cell transplant (HCT)

Introduction

Newborn screening (NBS) for Severe Combined Immunodeficiency (SCID) rapidly expanded across the United States (US) since 2010. By December 2018, all 50 states had

begun screening for SCID (1). SCID is not easily recognizable clinically at birth and is near uniformly fatal by 1 year of age without early treatment. Furthermore, immune function can be restored with life-saving hematopoietic cell transplantation (HCT), and survival is superior if HCT is performed in the first few months of life (2, 3). Finally, the absence or marked reduction of T cell receptor excision circles (TRECs) is highly associated with SCID, and TRECs are readily identifiable in the newborn dried blood spots (DBS) (4) of healthy newborns (5) thus SCID is particularly well suited for population-based NBS. In May of 2010, following reports of findings from earlier pilot studies in Wisconsin, the Navajo Nation, and our own statewide pilot SCID NBS program in Massachusetts, SCID was added to the US recommended uniform screening panel of conditions (6–8). This report presents a summary of findings from Massachusetts' first 10 years of SCID NBS experience, capitalizing on our ongoing data analyses for evidence-based quality improvement modifications to interpretation and notification protocols. We present our review of the diagnostic workup, in which we analyzed the relative value of flow cytometry and lymphocyte proliferation measurements for predicting a diagnosis of SCID or another genetic form of immunodeficiency. Additionally, we present TREC values linked to infants' gestational ages. We report the incidence of SCID in Massachusetts, the outcomes of definitive cellular therapy for the identified infants, and the diagnoses of additional infants with other conditions treated with cellular therapy.

Methods

The New England Newborn Screening Program (NENSP) developed and validated a high-throughput multiplex qPCR assay quantifying both the TREC analyte and an internal control (a single copy gene, RNaseP) in DNA extracted from a single DBS punch (9). The Commonwealth of Massachusetts authorized a statewide SCID NBS pilot that was initiated February 1, 2009 using a verbal consent protocol (105CMR270.010) yielding 98% enrollment; in 2016 SCID NBS became mandatory. Infants whose specimens showed low or absent TREC values were considered to have out-of-range SCID NBS results that required either a repeat NBS specimen or diagnostic testing of lymphocyte subsets with specified flow cytometry.

We routinely evaluated screening data and diagnostic outcomes using a baby-specific interpretation and reporting algorithm, that interpreted each individual test in the context of all other results from that specific baby. Our goal was to reduce the burden of positive screens while maintaining appropriate sensitivity. We developed a streamlined interpretation and notification protocol that discriminated between low and undetectable TREC values and that utilized evaluation of serial TREC values in neonatal intensive care unit (NICU) and non-NICU populations. Based on accruing data from the first two years of screening, we made three key evidence-based changes to our notification recommendations: 1) Initially, any infant with an out-of-range TREC result on any specimen (initial or repeat) was referred for lymphocyte subset testing. To avoid overwhelming primary care and NICU providers, families, and the small number of flow cytometry laboratories that met criteria to perform specialized pediatric diagnostic testing, we used data accrued among 16 infants referred during the first month of screening and changed the recommended action so that only infants with at least two out-of-range SCID NBS results would be referred for diagnostic testing by

flow cytometry. 2) After several months of screening (~110,000 infants), we observed that an undetectable TREC result on initial NBS specimens was both very infrequent (at the time, 0.01% of all infants screened; 4% of out-of-range screening results) and consistent with results of retrieved specimens tested from well-characterized SCID patients during our testing validation. We thus modified our interpretation and notification protocol to recommend immediate lymphocyte subset testing for any infant with an undetectable TREC result on an initial NBS specimen. 3) Simultaneously, we determined that 66 infants with serial NBS specimens who had at least one normal TREC result among multiple out-of-range TREC results had diagnostic results *inconsistent* with SCID. All 66 of these infants had prolonged NICU courses, often with complications including, but not limited to, extreme prematurity, cardiac defects, chylothorax, hydrops, gastrointestinal complications, and various genetic syndromes. We therefore modified our recommended-actions algorithm to allow us to release from follow up those infants who had serial specimens that included at least one normal TREC value.

To investigate the TREC values for premature infants, we evaluated data that were linked to infant gestational age at the time of specimen collection. For a subset of all specimens (infants from a three-month period in 2015), gestational age at birth was manually entered into a database. The difference between each infant's date of birth and date of specimen collection was then added to the gestational age at birth in order to calculate each infant's gestational age at the time of specimen collection.

Our current criteria for referral stipulates that infants are referred for testing of lymphocyte subsets by flow cytometry if they have undetectable TREC values on an initial NBS specimen *or* if they have out-of-range TREC values (<252 copies/ μL) on two serial specimens in the absence of a normal result. All infants requiring flow cytometry were sent for phlebotomy at one of multiple sites with a specialized requisition for lymphocyte subset testing that would occur within 24 hours. Diagnostic results were reported back to the NENSP for centralized tracking. Reference ranges for percent and absolute counts of lymphocyte populations were adapted from Shearer *et al* (10) and were standardized across the three sites that meet criteria for performing the testing. The minimal consensus panel included flow cytometry detecting surface expression of CD3, CD4, CD8, CD16 and/or CD56, CD19, and any of the following: CD45RA, CD45RO, CD62L, CD11a dim, CD31(9).

Normal flow cytometry was defined as ≥2500 T cells/μL (based on Shearer *et al*) *and* CD4 T cells >50% naïve *and* CD8 T cells >50% naïve. T-cell lymphopenia (TCL) was defined as having a CD3 <2500 cells/μL. The diagnosis of SCID was made according to criteria of the Primary Immune Deficiency Treatment Consortium (PIDTC), including typical SCID, leaky SCID, and Omenn syndrome (10). Other patients with TCL who did not meet PIDTC criteria for SCID but who had opportunistic infections, hypogammaglobulinemia, abnormal proliferation to phytohemagglutinin (PHA), poor antigen-specific T cell proliferation (e.g., tetanus and candida), and/or poor vaccine response were defined as combined immunodeficiency (CID). We calculated the risk of SCID or TCL and 95% confidence intervals.

Results

Overall Screening Outcomes

The NENSP screened 720,038 Massachusetts neonates (<30 days of age) for SCID between Feb 1, 2009 and January 31, 2019 (Figure 1). Out-of-range SCID NBS results were relatively infrequent (~0.29%). Of the 2,072 neonates who had at least one specimen with an out-of-range SCID NBS result, most (83%) were in a NICU or Special Care Nursery (SCN) at the time of specimen collection. Retrospective analyses of the 2,072 infants' data showed that only 237 neonates (11%, 0.03% of total screened) met criteria for referral for lymphocyte subset testing (Figure 1). Another 1,736 infants (84%) were released from follow-up testing by our algorithm as they had at least one independent NBS specimen with a normal TREC result that had been obtained either prior to the out-of-range TREC result (as is often the case with infants in NICUs) or on a subsequent requested repeat NBS specimen. An additional 92 neonates expired and 7 infants were lost to follow up prior to obtaining a repeat NBS specimen. While there was no way to rule out SCID definitively in these infants, none were thought to have had SCID, and all 92 deaths were attributable to causes common in NICU and premature infants.

Patients with undetectable TRECs on an initial specimen or with TRECs below cutoff on two occasions in the absence of ever having a normal result were referred for flow cytometry. Among the 237 neonates referred for lymphocyte subset testing, 35 were referred after having an undetectable TREC result on their initial NBS specimen, and the remaining 202 were referred after having two out-of-range SCID NBS results in the absence of a normal result.

TRECs and Gestational Age

Our initial data indicated lower TREC values among NICU infants than in the general neonatal population (9), but our evaluation did not distinguish premature infants from others in the NICU. Additionally, there was significant overlap in TREC values of the NICU and non-NICU populations. We analyzed 14,342 initial specimens with valid TREC results (normal or abnormal) received during a 3-month time frame that also had gestational age and date of specimen recorded on the filter paper lab slip (Figure 2). Similar to term neonates (n=13,455) for whom the rate of normal TREC value was 99.9%, neonates born prior to 37 weeks gestation (n=887) also had a high rate of normal TREC values (98%). Among the neonates who were born prior to 28 weeks gestation (n=42), 81% had normal TREC values.

Results of immunological follow-up

Lymphocyte subset data were available for 184 infants of the 237 referred infants (Figure 3). Of these 184 infants, 162 neonates (88%) were shown to have TCL and a single infant did not have TCL but was referred for evaluation based on a low naïve CD8 percentage. Infants with abnormal flow cytometry and an additional 6 patients without flow results were evaluated by immunologists. Nine neonates were diagnosed with SCID, 7 typical SCID and 2 leaky SCID (Table 2); the 9 included the first infant with SCID identified by SCID NBS in the United States (11). Seven infants were diagnosed with CID, 5 with genetically-defined

causes and 2 without. Three neonates had athymia (complete DGS (2), CHARGE syndrome (1)). There were 57 other infants identified with various syndromes known to be associated with TCL including 38 neonates with partial DGS. An additional 61 infants had secondary conditions to which TCL may be attributed. At this time, 32 infants appear to have idiopathic benign TCL. Of the 6 infants without flow results, 5 had diagnoses associated with TCL including DGS (1), Trisomy 21 (2), and chylothorax (1); the remaining infant was premature and had no other known diagnosis.

Impact of naïve/memory phenotyping and measurement of lymphocyte function

Our rationale for including naïve/memory phenotyping with flow cytometry was to ensure detection of cases of SCID with oligoclonal expansion or maternal engraftment, which may present with normal, falsely reassuring absolute T cell counts. We evaluated whether any of our cases of TCL were identified solely on the basis of abnormal naïve CD4 or CD8 T cell percentage; immunology referral was made solely on the basis of reduced percentage of naïve CD8 T cells in one case, and none were made based on reduced naïve CD4 T cells (Figure 4, Figure E1). The remaining 36 patients with low naïve T cell percentage would have been referred based on having low absolute T cell count.

Reduced naïve CD4 T cell percentage was associated with a higher likelihood that a patient with TCL would be identified as having SCID, athymia, or CID. Naïve CD4 T cell percentage was <50% in 14/17 infants (82%) with CID or complete DGS. In contrast, only 2/27 infants (7%) had a naïve CD4 T cell percentage <50% among infants with low TRECs that one might attribute to critical illness or treatment of prematurity (e.g. steroids). Infants with low TRECs attributed to partial DGS or critical congenital heart disease also included a minority of patients with naïve T cells <50% (6% and 19% respectively) (Figure 4). Similar results were seen in analysis of naïve CD8 T cells in these patient populations (Figure E1). The 5 premature infants with low naïve CD4 percentage (n=1), low naïve CD8 percentage (n=3) or both (n=1) had a median gestational age of 26 weeks, no different than the 22 infants with naïve percentage >50%, 26.9 weeks (p=0.9158).

Having undetectable TRECs was associated with a higher likelihood of the patient having SCID or TCL (Table 1). The risk that an infant with positive SCID NBS referred for flow would be diagnosed with SCID was 4% [95% CI: 1% – 6%] (in comparison to the general population risk of 0.001% [95% CI: 0.0004% – 0.002%]) and increased to 5% [95% CI: 0% – 15%] for an undetectable TREC in a NICU infant and to 47% [95% CI: 21% – 72%] for undetectable TREC in non-NICU infants. The risk of TCL was 88% [95% CI: 83% – 93%] for infants with positive NBS referred for flow, and increased to 100% and 93% for infants with undetectable TREC in NICU and non-NICU infants respectively.

Incidence of SCID and outcomes of transplantation

The incidence of SCID in Massachusetts in the 10-year period was ~1 in 80,000 (9 infants in 720,038 screened over 10 years (95% CI 1/48,000 to 1/240,000). All but one of the 9 infants had flow cytometry performed before 12 days of age and had genetic testing performed before 21 days of age (Table 2). All 9 infants have undergone definitive cellular therapy, either HCT or autologous gene therapy; most infants received definitive treatment without

any prior infections, and only 1 had active infection at time of transplant (Table 3). All 9 patients are alive with T cell reconstitution, and 7 of 9 are off of immunoglobulin replacement (Table 3). One infant with multiple intestinal atresia (MIA) was later found by research exome sequencing to have compound heterozygous *TTC7A* variants (12). After matched sibling HCT, he developed liver failure related to prolonged parenteral nutrition and had a successful multivisceral transplant at age 5 years.

In two cases, identification of an infant with SCID by NBS led to an additional unanticipated diagnosis in the family. The patient with *CD3D* SCID had had an older sibling born in Massachusetts prior to Massachusetts SCID NBS implementation, who had died out of the country. With consent, we retrieved the sibling's NBS specimen, which showed undetectable TREC values. The 5-year-old sibling of the patient with *JAK3* Leaky SCID, who had been born out of the country, came to medical attention in Massachusetts due to shingles and bronchiectasis, and was found to be affected with the same variants. This sibling ultimately underwent successful unrelated donor HCT and is alive and well, with excellent immune reconstitution.

Some infants identified with CID or athymia also underwent definitive cellular therapy. Two infants with CID of unknown genetic cause are now 2.2 and 2.8 years post-HCT. Of the two cases with complete DGS, one successfully underwent thymus transplantation without complications. The other case of complete DGS with confirmed 22q11 deletion and the athymic CHARGE syndrome case both expired before thymus transplantation could be performed, the latter due to cytomegalovirus (CMV) infection.

Discussion

Pilot newborn screening programs are typically implemented with conservative follow-up protocols that are later modified as screening and follow up data are accrued. Early in our screening experience as 1 of only 2 states conducting pilots of SCID screening, we pioneered two seminal interpretation and notification modifications based on our own empiric data to improve the overall efficiency and clinical effectiveness of the screening program. We modified our follow-up algorithm to require immediate lymphocyte subset testing for infants with an undetectable TREC result on an initial NBS specimen. Furthermore, we modified our recommendations such that infants with an out-of-range result and an independent specimen that showed a normal TREC result would not be referred for clinical evaluation, provided that the healthcare providers had no clinical concerns for SCID. The changes we made to our interpretation and notification algorithms ultimately decreased the number of infants requiring any further testing (by repeat NBS or flow cytometry) by 20%.

All NBS programs recognize that both NICU status and low gestational age yield a higher frequency of abnormal NBS results for many NBS conditions, and SCID is no exception to this. Eighty-three percent of infants with out-of-range SCID NBS results and 74% of infants prompting referral for lymphocyte subset testing were in a NICU. While NICU infants have a much higher rate of positive screening results compared to non-NICU infants, we believe that the NICU population, especially as it relates to prematurity, is at no lesser risk of having

SCID than the general population. In some cases, NICU status might be a risk factor for both SCID and other disorders associated with TCL that might be identified by the screen. In fact, of the 9 Massachusetts infants identified with SCID, two were NICU infants – one who passed through the NICU and one who was seriously ill. The latter infant presented with MIA, was being evaluated for Cystic Fibrosis (CF) (in-range CF NBS results) (12) and had a prolonged and difficult NICU course. SCID has been previously reported to be associated with MIA (13, 14), and this case demonstrates the importance of compliance with NBS follow up recommendations in NICU infants. Our follow up algorithm minimizes the burden on NICU teams and primary care providers while still recognizing the substantially increased risk of SCID in all infants with positive screening results.

While infants born prior to 37 weeks gestation had a lower median TREC value than those born at term, the median TREC value in premature infants was ~4x our pre-determined cutoff value for low TREC and the vast majority (98%) of premature infants had normal screening results. As expected, the frequency of normal NBS decreased from 99% (836/845) for specimens of infants aged 28 – 37 weeks to 81% (34/42) for infants aged < 28 weeks. Lymphopenia is common in preterm infants and affects all lymphocyte subsets, $CD4^+$ and $CD8^+$ T cells, B cells and NK cells, with infants at the lowest gestational age demonstrating the most severe lymphopenia (15). Premature (and NICU) infants routinely experience stressful stimuli that induce endogenous cortisol production (16). Both endogenous and exogenous corticosteroids reduce lymphocyte number and function (17, 18). Decreased concentration of IL-7 and reduced expression of IL-7 receptor in T cells of low gestational age infants has been proposed as another potential mechanism of the T cell lymphopenia (15). We nevertheless contend that the risk of SCID among premature and NICU infants with out-of-range TREC results is high enough to warrant the same timely follow up as infants born at term, and that follow up recommendations must be the same for all infants regardless of NICU status or gestational age. The algorithm, which selects urgent (undetectable TREC) and repeatedly abnormal TREC values, minimizes referral. Abnormal TREC results warrant attention in all infants.

With any screening test, there is cost to false positive results – in dollars, utilization of services, and patient/family anxiety. We therefore considered the impact of our cutoff for immunology referral of 2500 T cells/μL, which is higher than that of some other NBS programs, which use cutoffs of 1500 cells/μL. Of the 162 infants we identified with TCL, 44 had T cell counts between 1500 cells/μL and 2499 cells/μL, corresponding to an additional 4.4 cases per year referred for clinical evaluation. Of these 44 infants evaluated by immunologists, 36 did not have any other defined diagnosis explaining TCL (no cases of SCID, leaky SCID, or a monogenic CID), while the remaining 8 infants (18%) were found to have partial DGS. In 3 of these 8 cases, the diagnosis of DGS was made based on NBS (rather than congenital heart disease, other congenital anomalies, or hypocalcemia). How one weighs the benefit of making these diagnoses against the potential negative impact to patients and families who would otherwise not have been evaluated can be debated; for now, we plan to continue to evaluate patients with our current cutoff.

We examined the utility of naïve and memory T cell phenotyping as part of the flow cytometry panel. Over the 10-year period, measurement of naïve CD4 T cell percentage did

not lead to additional patient referral for formal immunology evaluation beyond absolute T cell count alone. However, our retrospective review indicates that patients with naïve CD4 T cells <50% were at far greater risk of having SCID or CID compared to those infants with naïve CD4 T cells >50% (relative risk 16, 95% CI: 5.8–46.1). We believe naïve T cell phenotyping therefore remains an important tool for risk stratification, along with absolute T cell counts and clinical history.

Massachusetts ten-year summary data revealed an incidence of SCID (1/80,000, [95% CI 1/48,000–1/240,000]) comparable to that observed in other states (19–23) and consistent with expectations for a rare disorder in a small screened population. We ensured quality data by a strict application of the case definition based on PIDTC criteria and statewide surveillance for potential misses through the MA SCID NBS Working Group, which has broad, statewide, multi-institutional, multidisciplinary representation, and no infants or children with false negative SCID NBS results have come to attention. Two children diagnosed with ZAP70 deficiency had normal TRECs at birth and low TRECs at the time of the diagnostic workup (at ages ~9 months and ~18 months) but this is expected (24–26).

Although our reported SCID incidence of 1/80,000 was comparable to the incidence of 1/125,000 [95% CI: 1/72,917–1/437,500] we observed in the pre-NBS era (7 known SCID infants born in Massachusetts between 1998–2008), the outcomes of the infants were different. All 7 were diagnosed as a result of opportunistic infection, and 2 had active infection at the time of HCT. One died before HCT, 2 died after HCT, and so only 4 of 7 survived (Table E1). In contrast, only 1 of the 9 infants identified by NBS had active infection and all 9 are alive post-transplant with excellent immune outcomes, a difference in survival which trends towards but does not meet significance (Fisher’s exact test, p=0.0625). We note that the median age at transplant was substantially lower, 6.5 months before NBS versus 3.4 months after NBS (Mann-Whitney test, p=0.0174). We are optimistic that the improvements we observed are likely to bear out with more years of screening. We attribute a good portion of our program’s success to effective coordination of screening, clinical follow-up, and treatment by the highly collaborative Massachusetts SCID NBS Working Group. Approximately 15% of 184 infants with lymphocyte subset data available were evaluated at more than 1 institution. The integration of NENSP, flow cytometry labs, and clinical experts in multiple disciplines is of vital importance for the success of SCID NBS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to acknowledge the work of the following people in the newborn screening molecular laboratory and follow-up section without whom this work would not have been possible: Jacalyn Gerstel-Thompson, Jolanta Kordowska, Samantha Barton, Lan Ji, Jennifer Navas, Claire Hughes and Annie Counihan. We would also like to acknowledge the Massachusetts Department of Public Health, which authorized the pilot program and operationalized routine SCID NBS through its relationship with the New England Newborn Screening Program of the University of Massachusetts Medical School. We also thank Dr. Barbara Stechenberg for her work as a member of the original SCID NBS workgroup and wish her the best in her retirement.

Funding information:

This work was funded in part by: the Center for Disease Control and Prevention 1U01EH000362-2008-2015 (to AMC).

LDN is supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

SYP was supported by the Translational Investigator Service at Boston Children's Hospital.

Abbreviations:

SCID	Severe combined immunodeficiency
NBS	newborn screen
TRECs	T cell receptor excision circles
HCT	hematopoietic cell transplant
DBS	dried blood spots

References Cited

1. Association of Public Health Laboratories. Severe Combined Immunodeficiency 2020 [Available from: https://www.newsteps.org/disorders/scid.
2. Pai S-Y, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation Outcomes for Severe Combined Immunodeficiency, 2000–2009. N Engl J Med. 2014;371.
3. Haddad E, Logan BR, Griffith LM, Buckley RH, Parrott RE, Prockop SE, et al. SCID genotype and 6-month posttransplant CD4 count predict survival and immune recovery. Blood. 2018;132(17):1737–49. [PubMed: 30154114]
4. Center for Disease Control and Prevention. Applying public health strategies to primary immunodeficiency diseases: a potential approach to genetic disorders. MMWR Morb Mortal Wkly Rep. 2004;53:1–29. [PubMed: 14724557]
5. Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. J Allergy Clin Immunol. 2005;115(2):391–7. [PubMed: 15696101]
6. Baker MW, Grossman WJ, Laessig RH, Hoffman GL, Brokopp CD, Kurtycz DF, et al. Development of a routine newborn screening protocol for severe combined immunodeficiency. J Allergy Clin Immunol. 2009;124(3):522–7. [PubMed: 19482345]
7. Comeau AM, Hale JE, Pai S-Y, Bonilla FA, Notarangelo LD, Pasternack MS, et al. Guidelines for implementation of population-based newborn screening for severe combined immunodeficiency. Journal of Inherited Metabolic Disease. 2010;33(Suppl 2):S273–81. [PubMed: 20490925]
8. Howell R Report on newborn screening for severe combined immunodeficiency: Secretary's Advisory Committee on Heritable Disorders in Newborns and Children. 2011.
9. Gerstel-Thompson JL, Wilkey JF, Baptiste JC, Navas JS, Pai S-Y, Pass KA, et al. High-throughput multiplexed T-cell-receptor excision circle quantitative PCR assay with internal controls for detection of severe combined immunodeficiency in population-based newborn screening. Clin Chem. 2010;56(9):1466–74. [PubMed: 20660142]
10. Shearer WT, Dunn E, Notarangelo LD, Dvorak CC, Puck JM, Logan B, et al. Establishing Diagnostic Criteria for SCID, Leaky SCID, and OMENN Syndrome: the Primary Immune Deficiency Treatment Consortium Experience. J Allergy Clin Immunol. 2013;133(4):1092–8. [PubMed: 24290292]
11. Hale JE, Bonilla FA, Pai S-Y, Gerstel-Thompson JL, Notarangelo LD, Eaton RB, et al. Identification of an infant with severe combined immunodeficiency by newborn screening. J Allergy Clin Immunol. 2010;126(5):1073–4. [PubMed: 20933257]

12. Chen R, Giliani S, Lanzi G, Mias GI, Lonardi S, Dobbs K, et al. Whole-exome sequencing identifies tetratricopeptide repeat domain 7A (TTC7A) mutations for combined immunodeficiency with intestinal atresias. J Allergy Clin Immunol. 2013;132(3):656–64.e17. [PubMed: 23830146]
13. Ali YAH, Rahman S, Bhat V, Al Thani S, Ismail A, Bassiouny I. Hereditary multiple intestinal atresia (HMIA) with severe combined immunodeficiency (SCID): a case report of two siblings and review of the literature on MIA, HMIA and HMIA with immunodeficiency over the last 50 years. BMJ Case Rep. 2011;2011:bcr0520103031.
14. Notarangelo LD. Multiple intestinal atresia with combined immune deficiency. Curr Opin Pediatr. 2014;26(6):690–6. [PubMed: 25268403]
15. Correa-Rocha R, Pérez A, Lorente R, Ferrando-Martínez S, Leal M, Gurbindo D, et al. Preterm neonates show marked leukopenia and lymphopenia that are associated with increased regulatory T-cell values and diminished IL-7. Pediatr Res. 2012;71(5):590–7. [PubMed: 22398700]
16. Mörelius E, He HG, Shorey S. Salivary Cortisol Reactivity in Preterm Infants in Neonatal Intensive Care: An Integrative Review. Int J Environ Res Public Health. 2016;13(3).
17. Ramaekers LH, Theunissen PM, Went K. Acute lymphopenia, stress, and plasma cortisol. Arch Dis Child. 1975;50(7):555–8. [PubMed: 1167069]
18. Zweiman B, Atkins PC, Bedard PM, Flaschen SL, Lisak RP. Corticosteroid effects on circulating lymphocyte subset levels in normal humans. J Clin Immunol. 1984;4(2):151–5. [PubMed: 6609933]
19. Chase NM, Verbsky JW, Routes JM. Newborn screening for SCID: three years of experience. Ann N Y Acad Sci. 2011;1238:99–105. [PubMed: 22129057]
20. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. Jama. 2014;312(7):729–38. [PubMed: 25138334]
21. Kwan A, Church JA, Cowan MJ, Agarwal R, Kapoor N, Kohn DB, et al. Newborn screening for severe combined immunodeficiency and T-cell lymphopenia in California: Results of the first 2 years. J Allergy Clin Immunol. 2013;132(1):140–1e7. [PubMed: 23810098]
22. Vogel BH, Bonagura V, Weinberg GA, Ballow M, Isabelle J, DiAntonio L, et al. Newborn screening for SCID in New York State: experience from the first two years. J Clin Immunol. 2014;34(3):289–303. [PubMed: 24578017]
23. Amatuni GS, Currier RJ, Church JA, Bishop T, Grimbacher E, Nguyen AA-C, et al. Newborn Screening for Severe Combined Immunodeficiency and T-cell Lymphopenia in California, 2010–2017. Pediatrics. 2019;143(2):51–63.
24. Grazioli S, Bennett M, Hildebrand KJ, Vallance H, Turvey SE, Junker AK. Limitation of TREC-based newborn screening for ZAP70 Severe Combined Immunodeficiency. Clin Immunol. 2014;153(1):209–10. [PubMed: 24797280]
25. Hauck F, Blumenthal B, Fuchs S, Lenoir C, Martin E, Speckmann C, et al. SYK expression endows human ZAP70-deficient CD8 T cells with residual TCR signaling. Clin Immunol. 2015;161(2):103–9. [PubMed: 26187144]
26. Jilkina O, Thompson JR, Kwan L, Van Caeseele P, Rockman-Greenberg C, Schroeder ML. Retrospective TREC testing of newborns with Severe Combined Immunodeficiency and other primary immunodeficiency diseases. Mol Genet Metab Rep. 2014;1:324–33. [PubMed: 27896105]

Highlights

1. **What is already known about this topic?** Universal newborn screening for severe combined immunodeficiency (SCID) by measuring T cell receptor excision circles (TRECs) in dried blood spots has been successful in many states and state-specific short-term outcomes (3–5 years) have been reported.
2. **What does this article add to our knowledge?** Our detailed immunologic and 10-year data reveals follow-up of infants with T cells <2500 cells/μL and/or low naïve T cell percentage, both premature and term, has high diagnostic yield. We present granular gestational age data.
3. **How does this study impact current management guidelines?** Referring NICU infants with out-of-range SCID newborn screen results regardless of gestational age and infants with mild lymphopenia (1500-2500 cell/μL) to immunologists is expected to lead to significantly improved outcomes for SCID patients.

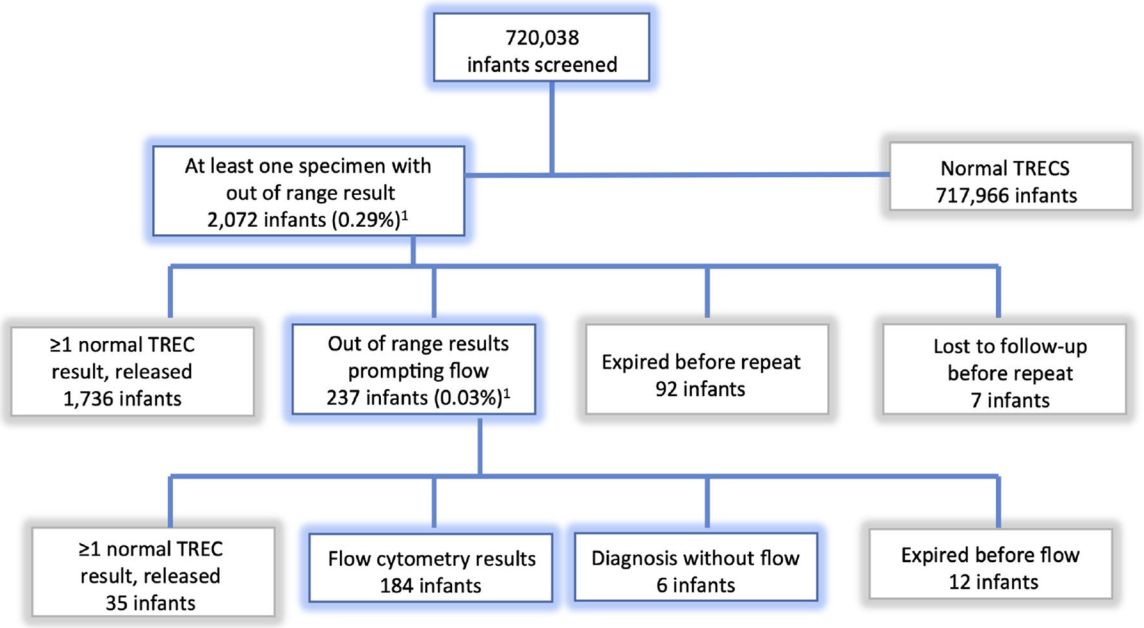

Figure 1.
Referral status for all infants evaluated by SCID NBS.

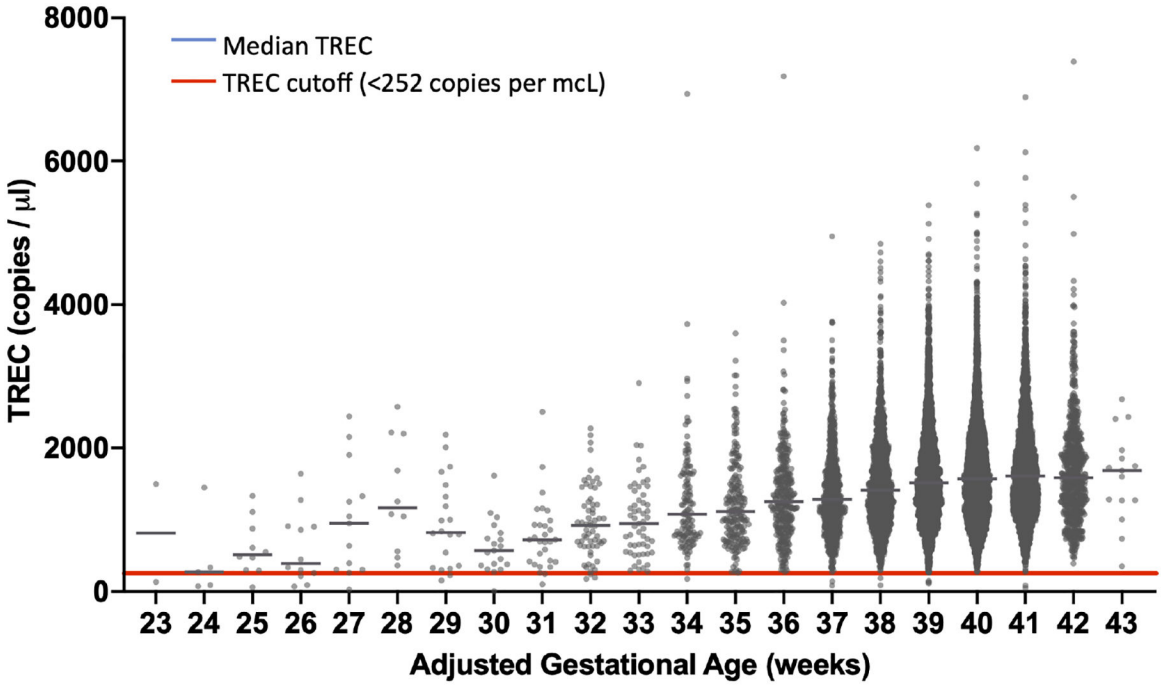

Figure 2.
TREC values by gestation age in 14,373 infants tested over a 3-month period.

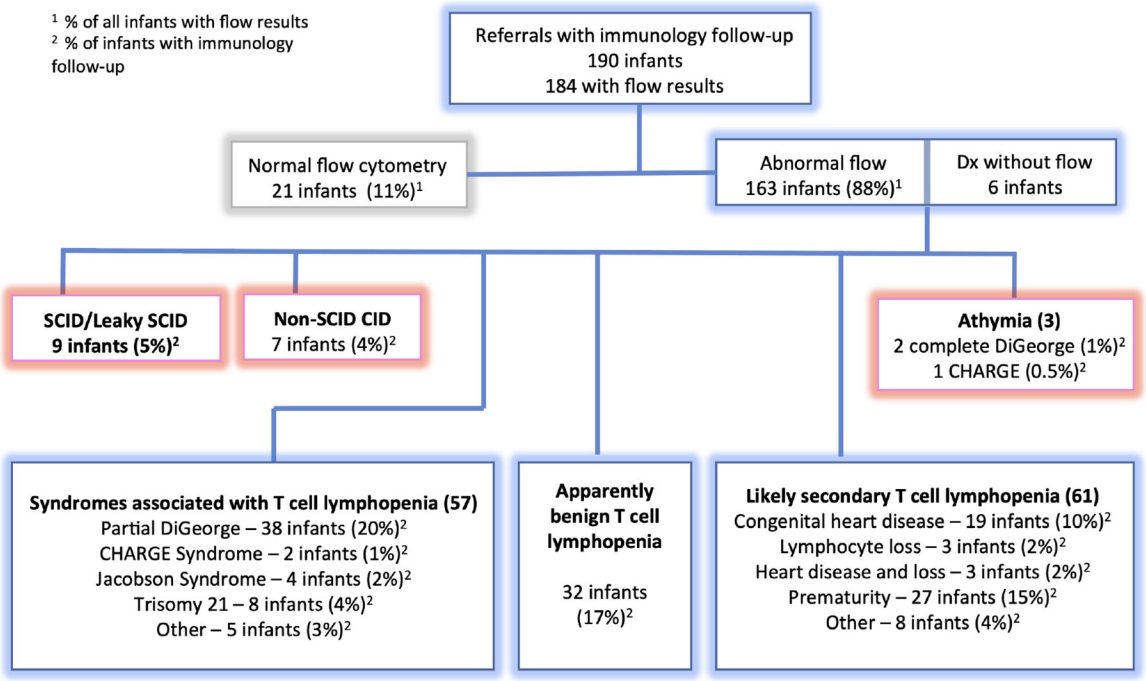

Figure 3.
Underlying cause of T cell lymphopenia diagnosed in infants with abnormal NBS.

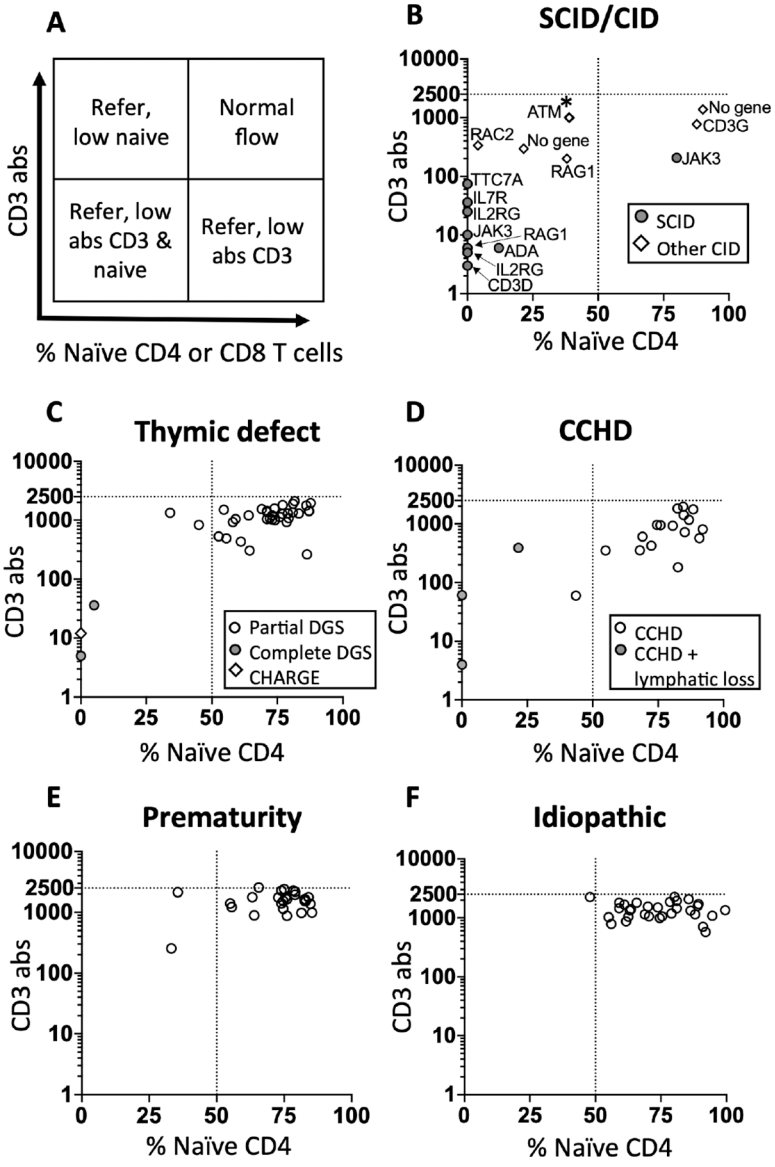

Figure 4.

A. Referral protocol based on absolute CD3 T cell count (CD3 abs) and %CD45RA^{+} (naïve) CD4 T and CD8 T cells. Patients were referred to immunology for CD3 abs < 2500 cells/mcL or naïve percentage of CD4 or CD8 T cells <50%. **B-F.** Absolute T cell counts and naïve CD4+ T cell percentages for patients with SCID/CID (B), thymic defects due to DiGeorge Syndrome or CHARGE syndrome (C), critical congenital heart disease (CCHD) (D), prematurity (born at < 37 weeks gestational age) (E), and patients with idiopathic T cell lymphopenia with normal *in vitro* T cell proliferation and no evidence of opportunistic infection or antibody deficiency (F). Dotted lines indicate cutoffs for CD3 abs and naïve CD4 T cells of 2500 cell/mcL and 50%, respectively. *Two ATM patients had nearly identical abs CD3 and % naïve CD4 T cells.

Table 1.

Risk of T cell lymphopenia (TCL) or SCID Relative to NBS Profile

NBS Profile	SCID Risk[#]			TCL Risk[*]		
	NICU	Not NICU	All	NICU	Not NICU	All
Undetectable TREC on Initial NBS	0.05 (1/20)	0.47 (7/15)	0.22 (8/35)	1.0 (14/14)	0.93 (14/15)	0.97 (28/29)
OOR but detectable TREC levels on two serial specimens in the absence of a normal result	0.006 (1/156)	0.0 (0/46)	0.005 (1/202)	0.84 (92/109)	0.91 (42/46)	0.86 (134/155)
Total	0.01 2/176	0.11 (7/61)	0.04 (9/237)	0.86 (106/123)	0.92 (56/61)	0.88 (162/184)

[*]TCL Risk calculated using denominator of infants within a particular NBS profile who had flow cytometry testing completed with TCL defined as CD3 <2500

[#]SCID Risk calculated using denominator of all infants within a particular NBS profile

Table 2:

Diagnostic features of Massachusetts infants diagnosed with SCID

Case #	Dx	Gene	TREC	Year of birth	Age at flow (days)	CD3 count	PHA (cpm)	PHA lower limit of normal	Age at genetic testing (days)	Age at genetic results (days)
594	SCID	JAK3	0	2010	6	10	2313	104415	10	42
935	SCID	TTC7A	<252	2011	52	74	2487	104415	79 *	n/a
1015	SCID	IL2RG	0	2011	12	5	739	104415	19	50
1050	SCID	CD3D	0	2011	11	3	507	104415	16	57
2053	leaky SCID	JAK3	0	2014	6	207	81209	104415	11	73
3023	SCID	ADA	0	2016	7	6	1233	96090	15	40
3840	SCID	IL2RG	0	2017	4	25	757	96090	12	33
4435	leaky SCID	IL7R	0	2018	11	36	13794	96090	11	35
4580	SCID	RAG1	0	2018	7	6	476	96090	15	27

* whole exome sequencing performed on a research basis

J Allergy Clin Immunol Pract. Author manuscript; available in PMC 2022 May 01.

Table 3:

Treatment characteristics and outcome of Massachusetts infants with SCID

Case #	Dx	Gene	Age at treatment (months)	Infectious Status at treatment	Donor	Conditioning	Years post	Status	Ig replacement status
594	SCID	JAK3	2.5	None	Unrelated HCT	MAC bu/cy	9.3	Alive	Off
935	SCID	TTC7A	3.4	None	Sibling HCT	ATG	8.3	Alive	On (post multivisceral transplant)
1015	SCID	IL2RG	2.4	None	Unrelated HCT	MAC bu/flu/ATG	8.2	Alive	Off
1050	SCID	CD3D	2.4	None	Unrelated HCT	MAC bu/flu/ATG	8.1	Alive	Off
2053	leaky SCID	JAK3	4.6	None	Unrelated HCT	MAC bu/flu/ATG	5.4	Alive	Off
3023	SCID	ADA	1.4	Aspergillus pneumonia, controlled	Sibling HCT	none	3.4	Alive	Off
3840	SCID	IL2RG	5.4	None	Autologous Gene Therapy	RIC bu	1.8	Alive	Off
4435	leaky SCID	IL7R	4.2	CMV viremia, active, pertussis treated	Unrelated HCT	Campath	1.0	Alive	On
4580	SCID	RAG1	4.0	None	Paternal haploidentical HCT	RIC bu/flu/thio/ATG	0.8	Alive	Off

(note: calculated to 1y post end of 10y screening period i.e. Jan 31, 2020)