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When Screening for Severe Combined Immunodeficiency (SCID) with T Cell Receptor Excision Circles Is Not SCID: a Case-Based Review

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Abstract

Newborn screening efforts focusing on the quantification of T cell receptor excision circles (TRECs), as a biomarker for abnormal thymic production of T cells, have allowed for the identification and definitive treatment of severe combined immunodeficiency (SCID) in asymptomatic neonates. With the adoption of TREC quantification in Guthrie cards across the

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USA and abroad, typical, and atypical SCID constitutes only ~ 10% of cases identified with abnormal TRECs associated with T cell lymphopenia. Several other non-SCID-related conditions may be identified by newborn screening in a term infant. Thus, it is important for physicians to recognize that other factors, such as prematurity, are often associated with low TRECs initially, but often improve with age. This paper focuses on a challenge that immunologists face: the diagnostic evaluation and management of cases in which abnormal TRECs are associated with variants of T cell lymphopenia in the absence of a genetically defined form of typical or atypical SCID. Various syndromes associated with T cell impairment, secondary forms of T cell lymphopenia, and idiopathic T cell lymphopenia are identified using this screening approach. Yet there is no consensus or guidelines to assist in the evaluation and management of these newborns, despite representing 90% of the patients identified, resulting in significant work for the clinical teams until a diagnosis is made. Using a case-based approach, we review pearls relevant to the evaluation of these newborns, as well as the management dilemmas for the families and team related to the resolution of genetic ambiguities.

Keywords

Newborn screening; idiopathic T cell lymphopenia; secondary T cell lymphopenia; syndromes associated with T cell lymphopenia

Introduction

Severe combined immunodeficiency (SCID) is a group of genetically heterogeneous primary immunodeficiency disorders (PIDDs) associated with abnormal T cell differentiation and/or function as well as varying degrees of abnormalities of B and NK cell development [1]. Quantification of T cell receptor excision circles (TRECs) from dried blood spots collected from every newborn in the United States (US) and other countries, is a biomarker for T cell receptor rearrangement and naïve T cells egressing from the thymus [2, 3]. Screening with the quantification of TRECs ensures that most newborns with typical and atypical SCID are diagnosed prior the onset of infections, allowing for rapid triage and institution of definitive therapies [2, 3].

It is of high importance that SCID is distinguished from those patients with athymia where thymic transplantation is preferred as definitive therapy. These two groups can be difficult to distinguish at birth based solely on clinical and immunological presentation. Genetic testing and sophisticated functional assays, such as the use of an in vitro artificial thymic environment, may provide a solution to this conundrum [4, 5]. The evaluation and management of newborns with a range of conditions other than SCID variants and athymia which are increasingly being identified because of these screening efforts, is a growing challenge for immunologists globally.

A recent report from the State of California documented the results of screening 3.2 million newborns using quantification of TRECs [6]. Most of the newborns with T cell lymphopenia were diagnosed with syndromes other than typical or atypical SCID (in decreasing order of frequency: DiGeorge syndrome, trisomy 21, ataxia telangiectasia, CHARGE syndrome, diabetic embryopathy, CLOVES syndrome, EXTL3 deficiency, Fryns syndrome, Nijmegen

syndrome, Noonan syndrome, and RAC2 deficiency). There were 28 newborns with true T cell lymphopenia and no identified disorder.

Despite the expansion and application of next generation sequencing (NGS) techniques including whole exome sequencing (WES) and whole genome sequencing (WGS), there remains a variety of non-SCID disorders that are associated with low TRECs and T cell lymphopenia identified as a result of newborn screening efforts, but without a clear genetic correlate [7, 8]. Similarly, other states, such as New York, reported on patients with idiopathic T cell lymphopenia identified by newborn screening for SCID [9]. One of the syndromes that was most recently associated with borderline abnormal TRECs is WHIM syndrome (warts, hypogammaglobulinemia, infections, and myelokathexis) for which targeted therapy is available. Therefore, for WHIM and other non-SCID lymphopenias, early diagnosis and treatment could improve clinical outcomes [10, 11]. Table 1 summarizes secondary conditions associated with non-SCID T cell lymphopenia that may be identified by newborn screening (NBS) for SCID. Tables 2 and 3 highlight the clinical and laboratory definition of SCID as well as the categorization of immunophenotype by genetic etiologies.

Immunologists may not be familiar with the diagnostic approach or the appropriate therapies available for rare disorders. Opportunities exist for immunologists to work together to develop evidence-based guidelines to assist clinicians in the diagnostic evaluation and management of these newborns [19]. With this goal in mind, we discuss evaluation and management of SCID and non-SCID cases that have been identified from the newborn screening program and pertain to the three following diagnostic categories: (1) syndromes associated with T cell impairment, (2) prematurity and secondary forms of T cell lymphopenia, and (3) idiopathic T cell lymphopenia. Additionally, we will briefly discuss challenges that have arisen in the context of the evaluation and treatment of these newborns, including those associated with the genetic ambiguities that often arise because of in-depth evaluations.

Syndromes Associated with T Cell Impairment

Various syndromes are frequently reported in association with abnormal TRECs and nonSCID T cell lymphopenia [7]. For the more common etiologies, such as 22q11.2 deletion syndrome or Trisomy 21, a careful and complete clinical exam coupled with a basic genetic evaluation including a karyotype and comparative genomic hybridization (CGH) array provides adequate clues to a timely and accurate diagnosis. For the rare etiologies, a delay in diagnosis may occur. Importantly, in some cases, a timely and accurate diagnosis is critical.

Case #1 An 8-day-old female was born at 34 2/7 weeks following a cesarean delivery weighing 2610 g. Maternal history included insulin dependent diabetes. The patient was observed to have facial dysmorphism, vertebral anomalies, bilateral hydronephrosis, and a patent ductus arteriosus and diffuse erythroderma. A positive newborn screen for SCID with a TREC = 0 copy/μL (reference range > 18 copies/μL) was reported on the newborn screen. Isolation and cessation of breast milk was instituted until the mother was tested for cytomegalovirus (CMV) status. On the complete blood count eosinophilia was present (1170 count/μL). The absolute lymphocyte count (ALC) was 2250 count/μL comprising CD3+

1417/μL, CD4+ 1298/μL, CD8+ 120/μL, CD4+CD45RA+ 12/μL (1% of CD4+ T cells), CD4+CD45RO+ 1223 (99%), CD8+ CD45RA+ 1 (1% of CD8+ T cells), CD8+CD45RO+ 116 (99%). However, the other lymphocyte subsets showed normal numbers (CD19+ 382/μL, and NK 264/μL). A karyotype was normal (46, XX) and a CGH array did not show 22q11.2. deletion. A primary immunodeficiency NGS panel, WES, and WGS did not identify any rare, or diagnostic pathogenic variants. Transient hypocalcemia and necrotizing enterocolitis occurred. After progression of the rash, increasing white blood cell count (WBC) (55,000/μL), adenopathy, and respiratory distress, corticosteroids were started. Immunological studies prior to treatment were notable for an IgG 814 mg/dL, IgM 79 mg/dL, IgA < 8 mg/dL, and IgE 1251 kU/L and diminished lymphocyte proliferation to phytohemagglutinin (PHA).

Prophylaxis included Bactrim, fluconazole, immunoglobulin replacement therapy (0.5 g/kg), and palivizumab to prevent respiratory syncytial virus (RSV) infection. Differential diagnosis still included both SCID and athymia-related conditions. CD34+ cells were isolated from peripheral blood and in vitro differentiation of CD34+ cells to T cells was normal supporting the diagnosis of primary athymia. Thymic transplantation was recommended.

The findings of remarkably normal appearing T cell counts in peripheral blood can be misleading, as these self-reactive T cells mainly have an activated phenotype (CD45RO), do not express naïve markers (CD45RA), and display an oligoclonal TCR Vβ repertoire. In this case, the clinical features of eosinophilia and a diffuse erythematous rash coupled with a predominance of memory T cells (99% of CD4+ and CD8+ T cells express CD45RO) suggested the possibility of Omenn syndrome (OS). OS-like features result from T cell infiltration of the skin causing erythroderma, as well as T cell infiltration, activation and expansion in the lymph nodes, gut, liver, and lung [20]. Although recombinase activating gene (*RAG1* and *RAG2)* hypomorphic variants were the first reported cause of OS; there are several other SCID gene variants linked to OS that result in defects involving metabolic pathways (*AK2*, *RMRP*, *ADA*), T cell signaling (*IL7RA*, *IL2RG*, *ZAP70*), V(D)J recombination [*DCLRE1C* (Artemis)], *LIG4* activity (DNA ligase IV), and thymic formation and function (partial DiGeorge syndrome with 22q11.2 deletion or *TBX1* mutation) and even CHARGE syndrome, with coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, and ear abnormalities [21–28]. In addition to OS, maternal lymphocyte engraftment with SCID or athymia was also considered [29]. DNA typing with variable number of tandem repeat (VNTR) loci was completed as a sensitive method to exclude maternal engraftment.

Athymia can be seen in CHARGE syndrome as well as within a variety of genetic disorders including 22q11.2 deletion syndrome (22qDS), *PAX1* deficiency, or biallelic mutations in *FOXN1* [30–32]. Dominant negative (heterozygous) mutations in *FOXN1* have also been associated with diminished thymic function in patients with and without nail dystrophy and alopecia [33]. Moreover, complete athymia in patients with FOXN1 deficiency may occur without concurrent hair and nail abnormalities [34]. In 22qDS, patients frequently present with congenital heart disease and hypoparathyroidism, but those findings are sometimes absent [35]. Patients with athymia typically have low or absent T cells as well as diminished

proliferative response to phytohemagglutinin [35]. In case #1, in addition to low numbers of overall T cells, proliferation to PHA was diminished. Because athymic patients can develop autoreactivity within their limited T cell compartment, immunosuppression using corticosteroids and calcineurin inhibitors, as well as other anti-T cell therapies, is often required as a bridge to thymic transplantation, which is curative [35, 36]. In a case like this, the timely decision to proceed with hematopoietic stem cell transplantation (HSCT) versus thymic transplantation is critical, so diagnostic efforts should not be delayed. Importantly, patients with athymia do not require HSCT, although in selected cases HSCT resulted in partial immune reconstitution but not cure [37].

The absence of naïve T cells is an important feature which is common to both SCID and athymia, and these can be identified by looking for presence of recent thymic emigrants that can be defined by CD45RA+CD31+ expression among CD4+ T cells. As we did not have a candidate gene on WES and WGS, we distinguished the possibility of athymia from SCID with a novel platform. Utilizing 5–10 mL of peripheral blood or bone marrow, research labs have developed methods to measure in vitro differentiation and positive selection of conventional human T cells from CD34+ cells using a variety of techniques including the use of an artificial thymic organoid system [4, 5]. Patients with isolated athymia as their only problem, can develop T cells in this assay, whereas those patients with other intrinsic defects of lymphocyte development do not.

As we described above, breastfeeding was discontinued in our patient to avoid exposure and infection with CMV from the mother. One important aspect of the management of syndromes associated with T cell impairment includes the issue of whether to withhold breastfeeding due to risk of transmission of maternal CMV [38, 39]. It is vital to consider the CD3+ T cell count and the percentage of naïve T cells present; however, the risks of transmission of CMV through breast milk in infants with T cell lymphopenia has not been studied in a large cohort allowing clinicians to safely identify a protective level of T cells [38]. Checking maternal CMV titers is vital to establish the risk of transmission prior to clearing further breast milk exposure and breastfeeding. Even in cases where mothers are seronegative for CMV, there have been cases of CMV transmission during maternal primary infection while breastfeeding.

Another important aspect of the management of syndromes associated with T cell impairment includes the question of whether to withhold live viral vaccines early in life. For newborns with CD3+ T cell counts < 1500/μL, vaccination with rotavirus is withheld due to concern for adverse events related to vaccination, such as vaccine-derived infections and associated diarrhea. There have been cases of PIDDs, including SCID, which were diagnosed after rotavirus vaccination caused severe gastroenteritis [40, 41]. The recommended schedule of childhood vaccines, except for live-viral vaccines, are typically provided safely to most newborns with syndromes associated with T cell impairment although they may be ineffective depending on the presence of residual immune function. PPSV23 (> 2 years of age) and meningococcal vaccines may also be considered. It is prudent to assure that vaccine responses to killed vaccines are carefully assessed by measuring titers 4–6 weeks following completion of the series.

Although our case was diagnosed with athymia that required immediate thymic transplant, many patients with partial thymic defects may continue without intervention. In these children, if there is an adequate T lymphocyte count and normal antigen response, vaccination with live viral vaccines including Varicella and MMR (not MMRV) may be considered. Adverse events associated with Varicella and MMR are uncommon in defined syndromes associated with T cell impairment or secondary T cell lymphopenia [42, 43]. The difference in recommendations between the Red Book and the Center for Disease Control (CDC) highlight the lack of uniformity in recommendations. Most studies in partial 22qDS document that vaccination with Varicella and MMR are safe with lower numbers of CD4+ T cells when accompanied by normal lymphocyte proliferation to mitogens [44]. MMR is safely administered in infants with CD4+ T cells > 500 cells/mm3. Similarly, other live vaccines including Bacille Calmette-Guerin (BCG) and polio virus have been associated with severe adverse events in the setting of PIDDs [45].

Prematurity and Secondary T Cell Lymphopenia

Prematurity and secondary T cell lymphopenia is frequently encountered in association with abnormal TRECs and non-SCID T cell lymphopenia. T cell lymphopenia is commonly documented among premature and low birth weight newborns [8, 46]. T cell lymphopenia in premature infants is part of the physiologic variation in thymus development. Frequently encountered clinical scenarios associated with secondary T cell lymphopenia include T cell loss which may occur because of lymphatic or gastrointestinal abnormalities [8, 47]. Rarely, other causes of secondary T cell lymphopenia, such as in utero exposures to maternal immunosuppressive agents, have also been described [8, 48].

Case #2 A 26-day-old male was born at 23 weeks weighing 540 g. He experienced multiple complications of extreme prematurity. A positive newborn screen for SCID with TREC = 10 (reference range > 18/μL) was documented. A complete blood cell count demonstrated an ALC of 2680/μL (ref range 2500–16,500). Flow cytometry demonstrated normal proportions of T, B and NK cells; however, the absolute number of T cells was documented at 1460/μL. Recent data provide lymphocyte subset reference ranges for premature infants. For this neonate, projected to be 27 weeks estimated gestational age, the median (5–95% CI) for absolute T cell counts is 1798/μL (900–3608). Furthermore, 78% of the CD4+ T cells and 74% of the CD8+ T cells expressed CD45RA, a marker identifying naive T cells, suggestive of adequate thymic function and production of T cells. These data were consistent with a diagnosis of T cell lymphopenia in association with prematurity, and no further immunological testing was advised. Standard care for age, clinical status, and degree of maturity of the newborn was recommended including routine childhood vaccination as well as live viral vaccination (e.g., rotavirus) when appropriate.

This case demonstrates that, in the setting of prematurity, it is vital to use recently published references ranges to correctly interpret lymphocyte immunophenotyping data among otherwise healthy preterm or low birthweight newborns [46]. Absolute numbers of T cells and B cells increase postnatally among preterm and low birthweight newborns; however, absolute numbers of NK cells appear to remain constant postnatally. Close follow-up of these preterm newborns may not be necessary; however, repeat lymphocyte

immunophenotyping and additional assessments of immune system function may be performed as dictated by the clinical scenario.

Secondary lymphopenia may develop with other conditions, as listed in Table 1. Neonatal complete thymectomy is associated with later reduction in T cell counts due to decreased production [49]. Moreover, the treatment of congenital heart disease resulting in thymectomy, thoracic duct pathology, and protein losing enteropathy may also be associated with lymphocyte loss [50, 51]. Underlying syndromes may also be associated with congenital heart disease alone or in combination with abnormal lymphatic development such as intestinal lymphangiectasia and chylothorax leading to lymphocyte loss [47]. Gastroschisis and intestinal atresia may also be associated with abnormal lymphatic development and lymphocyte loss. A careful and complete clinical examination often coupled with specific genetic evaluations can provide clues to the diagnosis. Moreover, in addition to the loss of lymphocytes, proteins may also be lost resulting in hypoalbuminemia and hypogammaglobulinemia, a clue that can often be overlooked. Close follow-up of these newborns may be necessary with repeat lymphocyte immunophenotyping once the underlying cause of lymphocyte loss is corrected.

Case #3 A 6-week-old infant was born at 39 weeks gestation weighing 3830 g. She had a prenatal diagnosis of gastroschisis and required intubation shortly after birth due to apnea. A positive newborn screen for SCID with TREC = 15 (reference range > 18/μL) was documented. The CBC showed an absolute lymphocyte count of 2290 cells/μL. The lymphocyte subsets were low with 1266 CD3+ T cells/μL (normal > 1512), 988 CD4+ T cells/μL (nl > 1025), and 208 CD8+ T cells/μL (nl > 400) (reference ranges per Amatuni et al. [6]). Naive, newly formed T cells (CD45RA+) comprised 61% and 61% of the CD4+ and CD8+ subsets, respectively, giving an indication of some new T cell generation in the thymus. B cell number was normal at 640 cells/μL, as was NK cell number at 202 cells/μL. Overall, these results were not considered normal but were sufficient to rule out typical SCID and were consistent with her diagnosis of gastroschisis. At that time, it was recommended that infectious precautions be implemented and that live vaccines be held; the remainder of vaccinations was carried out per normal scheduling. Repeat lymphocyte enumeration completed 2 months after her gastroschisis repair revealed normalization of the T cell compartment with normal mitogen proliferation.

In utero exposure to maternal immunosuppressive agents has also been documented as an important etiology of secondary T cell lymphopenia among newborns [48]. This underscores the need for a careful and complete clinical history of all newborns with non-SCID T cell lymphopenia. There have been cases with T cell lymphopenia due to in utero exposures to purine antagonists such as azathioprine and 6-mercaptopurine, as well as in utero exposures to anti-TNF alpha agents such as adalimumab. In these cases, the T cell lymphopenia typically resolved over time. However, these newborns may require consideration of supportive care measures such isolation, cessation of breast milk feeding, and prophylactic antibiotics including trimethoprim/sulfamethoxazole as well as avoidance of live viral vaccines due to the extremely low numbers of T cells.

Idiopathic T Cell Lymphopenia

Idiopathic T cell lymphopenia is encountered; however, its course remains variable [9]. Previous studies document that some newborns will have improvement fairly quickly whereas a subset of these newborns will not have appreciable improvement in T cell counts at all postnatally. A careful and complete clinical exam as well as a detailed laboratory assays, including the application of NGS typically fails to provide adequate clues to an underlying diagnosis in these cases. Careful clinical and laboratory reassessment is often necessary as a specific diagnosis may become apparent over time.

Case #4 A 7-day-old female was born at 39 weeks following a cesarean delivery weighing 3700 g. Maternal history was unremarkable. A preauricular skin tag with a normal hearing screen was documented. A positive NBS for SCID with a TREC = 5 (reference range > 18/μL) was documented. Isolation and cessation of breast milk was instituted. The ALC was 530/μL with CD3+ 172/μL, CD4+ 153/μL, CD8+ 3/μL, with preserved naïve T cell compartment (CD3+CD4+ CD45RA+ 125/μL (82%), CD3+CD8+CD45RA+ < 20/μL (71%)) and normal B lymphocyte and NK cell counts (CD19+79/μL, NK 243/μL). Mitogen proliferation to PHA was normal. Immunoglobulin levels demonstrated an IgG 817 mg/dL, IgM 13 mg/dL, and IgA < 8 mg/dL. Maternal engraftment was excluded using analysis of VNTR loci. A CGH array was negative for 22q11.2 deletion. A primary immunodeficiency NGS panel, WES, and WGS were all non-diagnostic. Prophylaxis included Bactrim, fluconazole, immunoglobulin, and palivizumab. At 41 days of age, a repeat ALC was 3770/μL with normalized T cell subsets (CD3+ 2357/μL, CD4+ 1863/μL, CD8+ 475/μL), CD19+519/μL, NK 791/μL, CD3+CD4+CD45RA+ 1496/μL (80%), CD3+ CD8+CD45RA+ 418/μL (88%). Supportive care was stopped, and the infant was discharged to home with outpatient follow-up.

Idiopathic T cell lymphopenia remains a diagnosis of exclusion. Care must be taken to carefully exclude previously aforementioned causes of T cell lymphopenia including syndromes associated with T cell impairment and secondary causes of T cell lymphopenia. In contrast to premature newborns whose T cell lymphopenia tends to improve postnatally, full-term newborns with idiopathic T cell lymphopenia may have persistently low T cell counts that do not improve over time. Follow-up of 26 newborns with idiopathic T cell lymphopenia documented that counts increased in 17 and decreased in 9, over a period of 6 to 66 months of follow-up. Another study of 33 newborns with idiopathic T cell lymphopenia documented that 13 resolved or improved, 13 were persistent and 6 became lost to follow-up [6, 9].

Ongoing evaluation of idiopathic T cell lymphopenia beyond the newborn period could include specific laboratory assessments such as a serum alpha fetoprotein at 6 months of age to help direct further evaluations for ataxia telangiectasia prior to the development of the classic features of progressive cerebellar ataxia and oculocutaneous telangiectasias [52]. In-depth genetic assessments using NGS techniques such as WES and WGS may be considered depending on the clinical scenario. Long-term follow-up and periodic reanalysis of sequencing data are recommended if lymphopenia persists. Recommendations regarding the extent of evaluation for individual patients and the duration of follow-up that is necessary

are unknown. Moreover, in this population, specific guidelines surrounding the safety of breastfeeding and vaccination with live viral vaccines including rotavirus vaccine remains uninvestigated and thus undefined. In addition, the safety and long-term impact of genetic testing in young infants is also unclear; genetic counseling should be a part of the evaluation if this is pursued.

Challenges: Screening, Genetics, and Ambiguities

The quantification of TRECs is a screening test that can detect most cases of typical and atypical SCID with only rare exceptions. Despite this, challenges arise when considering the evaluation of the non-SCID newborns with T cell lymphopenia identified by these screening efforts. Despite early identification, some families may refuse further workup for a variety of reasons including inconvenience, lack of understanding of the urgency for diagnosis and treatment, as well as denying the severity of the disorder. In addition, when we do continue investigations, they often create additional work for case managers and MDs trying to get authorizations or dealing with insurance denials, only to be challenged by the ambiguity of genetic sequencing data resulting in lengthy and laborious diagnostic evaluations carried out by multiple research labs. This scenario may lead to parental frustration and refusal of additional diagnostic evaluation.

Case #5 An 8-day-old male was born at 37 weeks of gestation following an uncomplicated pregnancy and vaginal delivery. Aside from hyperbilirubinemia and weight loss, facial dysmorphism as well as anomalies of the hands and toes suggested possible Trisomy 21. A positive NBS for SCID with a TREC = 1/μL (reference range > 18) was documented. Isolation and cessation of breast milk was instituted. The ALC was 550 /μL with CD3+154/μL, CD4+ 94/μL, CD8+ 56/μL, CD19+ < 20/μL, CD16/56+ NK cells < 20/μL, CD3+CD4+CD45RA+ 63/μL (67%), CD3+CD8+CD45RA+ 54/μL (96%). Mitogen proliferation to PHA was normal. Maternal engraftment was excluded using analysis of VNTR loci. A CGH array was negative for 22q11.2 deletion but did demonstrate changes consistent with a translocation form of Down syndrome. A primary immunodeficiency NGS panel also demonstrated one pathogenic variant in RAG1 and one variant of uncertain significance (VUS) in RAG1. Prophylaxis included Bactrim, fluconazole, immunoglobulin, and palivizumab. Additional testing to evaluate the pathogenicity of the heterozygous VUS in RAG1 was recommended. The family refused supportive care and additional laboratory evaluation as well as outpatient follow-up.

The increasing application of NGS has also led to challenges in the resolution of ambiguous results. Despite the application of bioinformatics, the ability to query databases, and the use of prediction tools focusing on a VUS, ambiguity still exists. Characterizing the segregation of the variant within the family as well as standardized reanalysis can be useful when evaluating a VUS. Collaboration with research laboratories or other professional groups of clinicians and scientists may support the ability to glean additional functional information about the VUS. For example, in the case above, flow cytometry can be used to detect utilization of the Va7.2 TCR gene segment in CD3+ T lymphocytes and/or mucosa-associated invariant T-169 cells (MAIT). This serves as a rapid method for evaluating

decreased usage of distal TCRaV (TRAV) gene segments, which suggests the presence of a underlying V(D)J recombination defect such as RAG deficiency [53].

Ultimately, however, the clinical phenotype coupled with laboratory assessments of immunologic function contribute to the decision to embark on curative therapies such as HSCT since intervention may need to occur before the results of additional testing (e.g., reanalysis) can provide more insight. In some cases, though, the exact genetic variant causing disease can be of high importance, especially in the era of personalized gene therapy. A recent publication categorized a novel variant initially classified as a VUS in two X-SCID patients identified by newborn screening. Functional assays and maternal X-inactivation studies were needed to confirm the pathogenicity of the novel variants [54]. In cases such as this, identification of the exact mutation is vital and can aid in planning for future therapies. Somatic mosaicism and somatic reversion are two other potential etiologies of T cell lymphopenia that might elude causal genetic analysis [55].

Conclusions

Immunologists continue to face challenges associated with the diagnosis and management of newborns with a range of conditions other than SCID which are increasingly identified because of these screening efforts. Familiarity with these common scenarios is vital to ensure that a thorough evaluation and appropriate management are provided. Creating a multiinstitutional collaborative to assist in the definition of best practices with respect to the diagnostic evaluation and management provided for these newborns will streamline the workup and diagnosis ensuring optimal treatment and even lifesaving therapies for individual patients.

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Table 1

Secondary conditions associated with non-SCID T cell lymphopenia that may be identified by NBS for SCID

Syndromes with T cell impairment
CHARGE syndrome (coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, and ear abnormalities)
Partial DiGeorge syndrome (congenital heart defects, hypoparathyroidism, and hypofunction of thymus)
VACTERL (vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistulas, renal and limb abnormalities)
Trisomy 21 (Down syndrome)
Ataxia-telangiectasia
Trisomy 18 (congenital heart defects and multiple anomalies)
Jacobsen syndrome (developmental delay, dysmorphic facies, abnormal bleeding, attention-deficit/hyperactivity disorder, and frequent ear and sinus infections)
CLOVES (congenital lipomatous [fatty] overgrowth, vascular malformations, and epidermal nevi and scoliosis)
Nijmegen breakage (microcephaly, hypogammaglobulinemia, decrease T cells, increased cancer risk, and abnormal DNA breakage repair)
Fryns (associated with congenital diaphragmatic hernia and dysmorphic features)
Ectrodactyly ectodermic dysplasia syndrome
EXTL3 deficiency (skeletal dysplasia, developmental delay)
Rac2 defect (neutrophil killing defects)
Noonan (dysmorphic facies, short neck, short stature, and congenital heart defects)
Renpenning (*PQBP1* loss of function leading to short stature, intellectual disability and dysmorphisms)
Barth syndrome (*TAZ* loss of function leading to cardiomyopathy at birth and neutropenia; mostly affects males)
TAR syndrome (*RBM8A* deficiency leading to thrombocytopenia and absent radius)
WHIM syndrome (*CXCR4* gain of function leading to warts, hypogammaglobulinemia, infections, myelokathexis)
Diabetic embryopathy
Other cytogenetic abnormalities (including metabolic diseases)
T cell loss or destruction
Congenital cardiac anomalies
Gastrointestinal anomalies (gastroschisis, omphalocele and intestinal lymphangiectasia)
Third spacing (anasarca, hydrops, and vascular leakage)
Neonatal leukemia
Other conditions
Maternal immunosuppressive medication (azathioprine and 6-mercaptopurine and adalimumab)
Extreme preterm birth (T cells normalize over time)
Idiopathic T lymphopenia

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* Table adapted from references [10, 12, 13]

Table 2

Definition of SCID variants based on Primary Immune Deficiency Treatment Consortium (PIDTC) criteria

Typical SCID	Leaky (Atypical) SCID	Omenn syndrome
Absence or very low number of T cells (CD3 T cells <300/uL) AND No or very low T cell function (< 10% of lower limit of normal) as measured by lymphocyte proliferative response to mitogen phytohemagglutinin (PHA) AND/ORM T cells of maternal origin present	Reduced number of CD3 T cells • for age up to 2 years < 1000/uL • for > 2 years up to 4 years < 800/uL • for > 4 years < 600/uL Absence of maternal engraftment < 30% of lower limit of normal T cell function (as measured by lymphocyte proliferative response to mitogen PHA Hypomorphic mutations in known SCID gene	Generalized skin rash Absence of maternal engraftment Detectable CD3 T cells, ≥ 300/mL Absent or low (up to 30% of normal) T cell proliferation to antigens to which the patient has been exposed If the proliferation to antigen was not performed, but at least 4 of the following supportive criteria, at least one of which must be among those marked with an asterisk (*) below, are present, the patient is eligible: • Hepatomegaly • Splenomegaly • Lymphadenopathy • Elevated IgE • Elevated absolute eosinophil count • *Oligoclonal T cells measured by CDR3 length or flow cytometry • *> 80% of CD3+ or CD4+ T cells are CD45RO+ • *Proliferation to PHA is reduced < 30% of lower limit of normal • *Proliferative response in mixed leukocyte reaction is reduced < 30% of lower limit of normal • *Mutation in SCID-causing gene

Adapted from references [14, 15]

Table 3

Genes linked to SCID and its variants and athymia presenting as SCID

	Immunophenotype			
	B+NK−	**B+NK+**	**B−NK+**	**B−NK−**
Genes affected	*IL2RG*	*IL7R*	*RAG1*	*AK2*
	JAK3	*IL2RB*	*RAG2*	*ADA*
	TBX1	*PTPRC*	*DCLRE1C (Artemis)*	*PNP*
		CD3D	*PRKDC*	
		CD3E	*NHEJ1*	
		CD3Z	*LIG4*	
		CORO1A		
		LAT		
		NFKB1		
		ZAP70		
		del22q11		

References [16–18]