Challenges of Newborn Severe Combined Immunodeficiency Screening Among Premature Infants

abstract

Newborn screening for severe combined immunodeficiency (SCID) is currently being performed in many states. It is important to address diagnostic challenges while outcomes are emerging from the first several years of screening. We present the case of a premature infant whose initial newborn screen was strongly positive for SCID. Subsequent lymphocyte subset analysis by flow cytometry was difficult to interpret due to the lack of age-matched reference values, a history of prenatal corticosteroid administration, and the possibility of maternal or posttransfusion engraftment. A repeat newborn screen for SCID ultimately revealed a normal result, confirming the initial newborn screen as a false positive. This case report reveals several of the diagnostic challenges unique to newborn SCID screening in premature infants and highlights the potential for states to address the feasibility of a standard protocol in this population. Pediatrics 2013;131:e1298–e1302

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KEY WORDS
newborn screening, severe combined immunodeficiency, premature infants

ABBREVIATIONS
ALC—absolute lymphocyte count
MDCH—Michigan Department of Community Health
SCID—severe combined immunodeficiency
TREC—T-cell receptor excision circle

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Severe combined immunodeficiency (SCID) is an inherited primary immunodeficiency syndrome characterized by a profound deficiency in T lymphocytes with variable defects in B-lymphocyte number and function. Recognition in the neonatal period is crucial because exposure to nonirradiated, nonleukoreduced blood products can result in life-threatening graft-versus-host disease or cytomegalovirus infection. Avoiding live vaccines and instituting strict isolation precautions are necessary because even common infections can be fatal.\(^1\) SCID meets many of the accepted criteria for newborn screening.\(^2\) Infants are classically asymptomatic at birth, SCID is fatal if untreated, and effective treatment with hematopoietic stem cell transplant is available with improved survival if performed at an earlier age.\(^3\)–\(^5\) The screen involves quantification of T-cell receptor excision circles (TRECs), which are nonreplicative pieces of DNA formed during T-cell receptor gene rearrangement in the thymus.\(^6\) Among the states performing TREC screening, there have been no published reports to date of patients diagnosed with SCID who were missed by the screen.\(^1\)

There have now been a few publications reporting higher false-positive TREC screening rates among premature infants. Lymphocyte enumeration by flow cytometry is often performed as confirmatory testing for SCID, although there is variation among states regarding the management of positive TREC screens in premature infants.\(^6,7\) These infants pose particular difficulty with interpretation of flow cytometry, due to the lack of age-matched reference values and frequent administration of prenatal corticosteroids.\(^7\)–\(^9\) We present the case of a premature infant whose initial TREC screen was strongly positive for SCID. This case reveals several of the challenges associated with TREC screening in premature infants.

**PATIENT PRESENTATION**

The patient was a white female born at gestational age 33 weeks with a birth weight of 2090 g. The infant was the product of a monochorionic-triamniotic triplet gestation. The pregnancy was complicated by feto-fetal transfusion syndrome. Prenatal betamethasone was administered on 2 occasions. The immediate postnatal course was complicated by hydrops fetalis and hypoxemia with a hematocrit of 18.9%. The first newborn screen was collected before any blood product transfusion at 2 hours of life, in accordance with the Michigan Department of Community Health (MDCH) Newborn Screening Guide. The infant subsequently received a transfusion with leukoreduced, nonirradiated, O-negative red blood cells. A second newborn screen was obtained 24 hours after transfusion, in compliance with the MDCH guidelines (Fig 1).\(^10\)

On day of life 4, the infant developed acute hemodynamic compromise and received multiple leukoreduced, nonirradiated, cross-matched red blood cell transfusions. She was suspected of having sepsis and empirically started on vancomycin and gentamicin. The absolute lymphocyte count (ALC) reached a nadir of 1200 cells per \(\mu\)L. Antibiotics were discontinued after 48 hours because the blood culture remained negative. On physical examination, the infant was sedated, intubated, and receiving high-frequency oscillatory ventilation. She had generalized edema. No skin rashes were noted. Hospital medications included hydrochlorothiazide, hydrocortisone, lorazepam, and morphine.

On day of life 9, the first newborn screen result was received and notable for a very low TREC value of 3 (Table 1), indicating a strong positive for SCID or other primary immune deficiency. The threshold in Michigan for an abnormal TREC screen in full-term infants was <30. The parents were informed of the abnormal screen indicating that their infant may have a severe immunodeficiency and potentially require a bone marrow transplant. The infant was placed under strict isolation precautions until additional testing was performed. According to the MDCH protocol, the decision to pursue flow cytometry as confirmatory testing was determined on an individual basis after evaluation by the local referral center (in this case, the University of Michigan). Flow cytometry was performed on day of life 9.

Flow cytometry results were obtained on day of life 10 and initially suggested a possible T-cell primary immunodeficiency. The interpretation was complicated by prenatal and postnatal steroid administration, previous

**FIGURE 1**

Michigan Department of Community Health newborn screening guidelines for newborns transfused with red blood cells after first newborn screen collection.
nonirradiated blood transfusions, and the possibility of maternal engraftment. The T-cell (CD3+), helper T-cell (CD3+CD4+), and cytotoxic T-cell (CD3+CD8+) counts were below the reported normal ranges. However, memory helper T-cell (CD3+CD8+) and memory cytotoxic T-cell (CD3+CD8+) lymphocyte percentages were above normal and within the normal range, respectively (Table 2). The possibility of maternal or posttransfusion engraftment was considered, although a more profound trend toward memory T lymphocytes as compared with naive T lymphocytes would have been expected.

The result of the second newborn screen collected 24 hours after the initial transfusion was reported on day of life 11 (Table 1). Of note, previous transfusions are not thought to affect the TREC screen. The result was reported as inconclusive due to inadequate β-actin DNA control (personal communication, MDCH August 2012). Although not part of the MDCH algorithm, a third newborn screen was collected on day of life 14 because other states’ health departments have recommended repeating positive screens in premature infants until the screen is normal or until the infant reaches 37 weeks’ gestation.6,7 The third newborn screen ultimately revealed a normal TREC number (Table 1). Flow cytometry was not repeated, because the first newborn screen was presumed to be a false positive. Notably, chest radiography revealed the presence of a thymic shadow. Family history was negative for immunodeficiency, miscarriages, or infant deaths. The infant’s clinical course remained complicated due to respiratory and feeding difficulties, although there was no clinical suspicion of impaired immune function or opportunistic infection.

**DISCUSSION**

Newborn screening for SCID involves enumeration of TREC, a surrogate marker for circulating naive T lymphocytes.7 Screening thereby results in the identification of a variety of T-cell lymphopenias, such as that associated with 22q11.2 deletion syndrome, in addition to the classic SCID phenotype. An abnormal TREC screen may also represent secondary T-cell defects, such as congenital heart disease, neonatal leukemia, lymphangiectasia, metabolic defects, or lymphocyte extravasation.6,7,11 In our patient, hydrops fetalis may have contributed to the initial abnormal TREC screen due to extravasation of lymphocytes.

Confirmatory testing for SCID involves flow cytometry, which may be costly when considering widespread implementation. Standard flow cytometry costs >$400, whereas repeat TREC assays cost ~$5.12 As in our patient, flow cytometry may be challenging to interpret in premature infants due to the possibility of maternal engraftment. Maternal T lymphocytes have been detected in healthy infants and infants with SCID, revealing that the placenta is an incomplete barrier. In immunocompetent newborns, maternally derived T lymphocytes are detected and eliminated. In infants with SCID, the inability to reject maternal cells can lead to engraftment. The incidence of maternal engraftment among infants with SCID has been reported to be between 25% and 40%.15–16

The combination of zero TREC and <200 cells per μL T lymphocytes is generally accepted as diagnostic for SCID, although any ALC <2500 cells per μL is potentially representative of lymphopenia in early infancy.6,17 In our patient, the ALC reached a nadir of 1200 cells per μL on day 4. In addition, the reference ranges listed in Table 2 were determined from full-term infants, because no such normal values exist for premature infants.17 Nevertheless, several findings argue against this infant having SCID, including the presence of a thymic shadow on chest radiography, flow cytometry that was not consistent with classic SCID or SCID with maternal engraftment, and a history of hydrops fetalis.

**TABLE 1** TREC Screening Results Arranged by Age

<table>
<thead>
<tr>
<th>Corrected Gestational Age</th>
<th>Day of Life</th>
<th>TREC Value</th>
<th>TREC Screening Result Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>33 wk and 4 d (day of birth)</td>
<td>1</td>
<td>3</td>
<td>Strongly positive</td>
</tr>
<tr>
<td>33 wk and 6 d</td>
<td>3</td>
<td>Inadequate β-actin DNA control</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>35 wk and 3 d</td>
<td>14</td>
<td>&gt;30</td>
<td>Within normal limits</td>
</tr>
</tbody>
</table>

**TABLE 2** Primary Immunodeficiency (SCID) Panel (T Cell, B Cell, Natural Killer Cell, and Memory/Naive T Lymphocyte Subset Quantitation by Flow Cytometry)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Description</th>
<th>Percentage of Lymphocytes (Reference Range)a,b</th>
<th>Absolute Cell Count (Reference Range) c,c</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>T cell</td>
<td>43.4 (55–84)</td>
<td>1625 (2500–5500)</td>
</tr>
<tr>
<td>CD3*</td>
<td>Helper T cell</td>
<td>36 (35–64)</td>
<td>1546 (1800–4000)</td>
</tr>
<tr>
<td>CD3*8+</td>
<td>Cytotoxic T cell</td>
<td>6.1 (12–28)</td>
<td>230 (580–1700)</td>
</tr>
<tr>
<td>CD3*16/56+</td>
<td>Natural killer cell</td>
<td>37.1 (4–18)</td>
<td>1389 (1170–1100)</td>
</tr>
<tr>
<td>CD19</td>
<td>B cell</td>
<td>8.1 (6–32)</td>
<td>305 (300–2000)</td>
</tr>
<tr>
<td>CD19*</td>
<td>Naive helper T cell</td>
<td>63.7 (64–95)</td>
<td>951 (1200–3700)</td>
</tr>
<tr>
<td>CD19*45RO+</td>
<td>Memory helper T cell</td>
<td>29 (2–22)</td>
<td>434 (60–900)</td>
</tr>
<tr>
<td>CD19*8 45RO+</td>
<td>Naive cytotoxic T cell</td>
<td>93.2 (80–99)</td>
<td>314 (450–1500)</td>
</tr>
<tr>
<td>CD19*16 45RO+</td>
<td>Memory cytotoxic T cell</td>
<td>5.1 (1–5)</td>
<td>19 (50–350)</td>
</tr>
</tbody>
</table>

a Values represent percentages.
b Values represent cells/μL.
c Values represent cells/μL.

d Baby's reference ranges are from J Allergy Clin Immunol 2003;112(5):973–980.17
This case supports previous findings that initially positive TREC screens from premature infants may be false positives. In Wisconsin, where TREC screening has been performed the longest, the observed high false-positive rate in premature infants led to the standard practice of repeating positive TREC assays until the assay is normal or until the infant reaches an adjusted gestational age of $\geq 37$ weeks. By using this algorithm, the repeat testing rate was below that of other newborn screen assays. The higher false-positive rate in premature infants may relate to the lack of standard TREC values in this age group. Although there are no population-based studies addressing normal TREC values in premature infants, a study estimated that the TREC value increases 9.8% per week of gestation. Many premature infants, including our patient, are exposed to prenatal and occasionally postnatal corticosteroids, which may confound results of screening or flow cytometry. Controversy exists regarding prenatal steroid administration and its effect on fetal lymphocyte cell counts. A prospective study evaluating the effect of prenatal corticosteroids found that there were significantly fewer ALC, CD4, and CD25 subset populations even after adjustment for gestational age. In contrast, a study designed to evaluate infants with abnormal TREC screens who died before immune testing revealed no significant correlation between TREC value and exposure to prenatal steroids.

The TREC assay is a screening test, not a diagnostic test. Similar to other newborn screening tests, allowance of some false-positive results is acceptable because a low rate of false-negative results is necessary to avoid missing cases of a rare but serious disease. Although the false-positive result in premature infants may be transient, this can cause undue anxiety among parents and generate confusion among practitioners. There are likely many cases of false-positive SCID screens in premature infants similar to the case presented, but practitioners need to remain cognizant that premature infants with positive screens may have true SCID. It is important to administer leukoreduced, irradiated blood products as necessary and to initiate strict isolation precautions until further testing differentiates true SCID from secondary causes of abnormal TREC screens. Flow cytometry can be challenging to interpret in this population, and treatment with bone marrow transplant cannot be performed until infants are healthy. It therefore seems reasonable to repeat the TREC screen until it is normal or until the infant reaches 37 weeks gestation. Although there is considerable variability among states regarding newborn SCID screening, additional studies addressing the feasibility of standard algorithms in the premature infant population may facilitate a more uniform approach to optimize outcomes and costs.

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