The Benefits of Second NBS to Detect SCID and Other T-cell Lymphopenias (TCL): A Four-Year Review in Washington State

Jay Patel, MD (Primary Co-Author)
Caroline T. Nucup-Villaruz, MD (Primary Co-Author and Presenter)
Suzanne Skoda-Smith, MD, Troy Torgerson, MD, Tim Davis, Benjamin Peprah, Arun Singh and John Thompson, PhD

NBS and Genetic Testing Symposium - April 8, 2019, Chicago, IL
NBS for SCID in Washington State: Cut-off and Protocol

- SCID (TREC) screening began in WA on January 2, 2014
- Result Classification: TREC ≥ 81 - NORMAL
  TREC ≤ 80 - BORDERLINE
  TREC ≤ 60 - PRESUMPTIVE
- 1st NBS Presumptive → Referral, recommend flow cytometry
- 1st NBS Borderline → wait/request 2nd NBS
- Persistently borderline TREC on 1st & 2nd NBS → Referral, recommend flow cytometry
- Subsequent (3rd or 4th NBS) borderline or presumptive TREC → Referral, recommend flow cytometry
1st Newborn Screen
(18 – 48 hours of life)

Normal (TREC > 80)

Borderline (TREC 61 – 80)

Presumptive (TREC ≤ 60)

Referral to Immunologist made, diagnostic flow cytometry obtained
Washington State SCID Screening Algorithm

1st Newborn Screen
(18 – 48 hours of life)

2nd Newborn Screen
(7 – 14 days of life)

- Normal (TREC > 80)
- Borderline (TREC 61 – 80)
- Presumptive (TREC ≤60)

- Not obtained
  - Normal (TREC > 80)

- Case closed, Referral not made

- Referral to Immunologist made, diagnostic flow cytometry obtained
Washington State SCID Screening Algorithm

1st Newborn Screen
(18 – 48 hours of life)

2nd Newborn Screen
(7 – 14 days of life)

- Normal (TREC >80)
  - Normal (TREC >80)
  - Borderline (TREC 61 – 80)
  - Presumptive (TREC ≤60)

- Borderline (TREC 61 – 80)
  - Normal (TREC >80)
  - Borderline (TREC 61 – 80)
  - Presumptive (TREC ≤60)

- Presumptive (TREC ≤60)
  - 3rd NBS obtained, consult with Immunologist
  - Referral not made
  - Referral to Immunologist made, diagnostic flow cytometry obtained
  - Case closed, Referral not made
Newborn Screening Timeline

First newborn Screen (18-48 hours) → Second Newborn Screen (7-14 days) → DOH Referral to SCH Immunology / Flow cytometry Requested → Flow cytometry resulted → Intervention: SCID Safety Script, Follow-up labs (PHA), Immunology clinic visit, HSCT
Confirmatory Testing

• Confirmatory testing sent to Seattle Children’s Lab
  • Includes total WBC, differential, CD3/4/19/16/56/DR lymphocyte counts, CD4CD45RA+RO- percentage
  • Uses 1-2mL whole blood

• Interpretation:

<table>
<thead>
<tr>
<th>Abnormal confirmatory screen</th>
<th>CD3 ≤ 1500 and/or CD4CD45RA ≤ 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equivocal confirmatory screen</td>
<td>CD3 &gt; 1500 and CD4CD45RA &gt; 20%</td>
</tr>
<tr>
<td>False positive</td>
<td>CD3 ≥ 2500 and abnormal TREC</td>
</tr>
</tbody>
</table>

• ~40% of centers in US from *PIDTC survey assess CD4CD45RA+RO- percentage

*PIDTC – Primary Immune Deficiency Treatment Consortium
Confirmatory Testing

- Confirmatory testing sent to Seattle Children’s Lab
  - Includes total WBC, differential, CD3/4/19/16/56/DR lymphocyte counts, CD4CD45RA+RO- percentage
  - Uses 1-2mL whole blood

- Interpretation:

<table>
<thead>
<tr>
<th>Abnormal confirmatory screen</th>
<th>CD3 ≤ 1500 and/or CD4CD45RA ≤ 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equivocal confirmatory screen</td>
<td>CD3 &gt; 1500 and CD4CD45RA &gt; 20%</td>
</tr>
<tr>
<td>False positive</td>
<td>CD3 ≥ 2500 and abnormal TREC</td>
</tr>
</tbody>
</table>

- ~40% of centers in US from PIDTC survey assess CD4CD45RA+RO- percentage
### Referrals based on sequence of NBS (2014-2017)

<table>
<thead>
<tr>
<th>1st NBS Referral</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>True SCID</td>
</tr>
<tr>
<td>1</td>
<td>Leaky SCID</td>
</tr>
<tr>
<td>8</td>
<td>Idiopathic TCL</td>
</tr>
<tr>
<td>8</td>
<td>Syndromes with T-cell impairment</td>
</tr>
<tr>
<td>9</td>
<td>Secondary TCL</td>
</tr>
<tr>
<td>1</td>
<td>Preterm</td>
</tr>
<tr>
<td>10</td>
<td>False Positive</td>
</tr>
<tr>
<td>42</td>
<td>TOTAL</td>
</tr>
</tbody>
</table>

First newborn Screen (18-48 hours) → DOH Referral to SCH Immunology / Flow cytometry Requested → Flow cytometry resulted → Intervention: SCID Safety Script, Follow-up labs (PHA), IMM Clinic, HSCT

21.5 hours 5.6 days 7 days

Average age of newborn from screening, release/reporting of results to follow-up action
Referrals based on sequence of NBS (2014-2017)

<table>
<thead>
<tr>
<th>Category</th>
<th>2nd NBS Referral</th>
</tr>
</thead>
<tbody>
<tr>
<td>True SCID</td>
<td>0</td>
</tr>
<tr>
<td>Leaky SCID</td>
<td>0</td>
</tr>
<tr>
<td>Idiopathic TCL</td>
<td>0</td>
</tr>
<tr>
<td>Syndromes with T-cell impairment</td>
<td>5</td>
</tr>
<tr>
<td>Secondary TCL</td>
<td>7</td>
</tr>
<tr>
<td>Preterm</td>
<td>0</td>
</tr>
<tr>
<td>False Positive</td>
<td>7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>19</td>
</tr>
</tbody>
</table>
### Referrals based on sequence of NBS (2014-2017)

<table>
<thead>
<tr>
<th>1st NBS Referral</th>
<th>Category</th>
<th>2nd NBS Referral</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>True SCID</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Leaky SCID</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Idiopathic TCL</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Syndromes with T-cell impairment</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>Secondary TCL</td>
<td>7</td>
</tr>
<tr>
<td>1</td>
<td>Preterm</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>False Positive</td>
<td>7</td>
</tr>
<tr>
<td>42</td>
<td>TOTAL</td>
<td>19</td>
</tr>
<tr>
<td>Category</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>True SCID</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Leaky SCID</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Idiopathic TCL</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Syndromes with T-cell impairment</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Secondary TCL</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Preterm</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>False Positive</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>14</strong></td>
<td></td>
</tr>
</tbody>
</table>
### SCID and TCL: Jan 2014 to Dec 2017

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>True SCID</td>
<td>5 (8%)</td>
</tr>
<tr>
<td>Leaky SCID</td>
<td>1 (1%)</td>
</tr>
<tr>
<td><strong>TCL, no known cause (CD3: 300 - 2500)</strong></td>
<td>11 (15%)</td>
</tr>
<tr>
<td>• CD3: 300-1500 (abnormal)</td>
<td>5</td>
</tr>
<tr>
<td>• CD3: 1500-2500 (equivocal)</td>
<td>6</td>
</tr>
<tr>
<td>Syndromes with T-cell impairment</td>
<td>15 (20%)</td>
</tr>
<tr>
<td>Secondary TCL</td>
<td>22 (29%)</td>
</tr>
<tr>
<td>Preterm</td>
<td>3 (4%)</td>
</tr>
<tr>
<td><strong>False Positive (CD3 ≥ 2500)</strong></td>
<td>18 (24%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>75</td>
</tr>
</tbody>
</table>
Total SCID Data: Jan 2014 to Dec 2017

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive screen or Low TREC (not referred, resolved by 2nd or subsequent NBS)</td>
<td>160</td>
</tr>
<tr>
<td>Cases Referred but Excluded</td>
<td>15</td>
</tr>
</tbody>
</table>
### Total SCID Data: Jan 2014 to Dec 2017

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive screen or Low TREC (not referred, resolved by 2nd or subsequent NBS)</td>
<td>160</td>
</tr>
<tr>
<td>Cases Referred but Excluded</td>
<td>15</td>
</tr>
<tr>
<td>Referrals:</td>
<td></td>
</tr>
<tr>
<td>Based on 1(^{st}) NBS</td>
<td>42</td>
</tr>
<tr>
<td>Based on 2(^{nd}) NBS</td>
<td>19</td>
</tr>
<tr>
<td>Based on 3(^{rd}) or 4(^{th}) NBS</td>
<td>14</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75</td>
</tr>
<tr>
<td>True (Typical) SCID plus Leaky SCID</td>
<td>6</td>
</tr>
<tr>
<td>TCL (idiopathic, secondary, syndromes)</td>
<td>48</td>
</tr>
<tr>
<td>Preterm</td>
<td>3</td>
</tr>
</tbody>
</table>
## Total SCID Data: Jan 2014 to Dec 2017

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive screen or Low TREC (not referred/resolved)</td>
<td>160</td>
</tr>
<tr>
<td>Cases Referred but Excluded</td>
<td>15</td>
</tr>
<tr>
<td><strong>Referrals:</strong></td>
<td></td>
</tr>
<tr>
<td>Based on 1\textsuperscript{st} NBS</td>
<td>42  (56%)</td>
</tr>
<tr>
<td>Based on 2\textsuperscript{nd} NBS</td>
<td>19  (25%)</td>
</tr>
<tr>
<td>Based on 3\textsuperscript{rd} or 4\textsuperscript{th} NBS</td>
<td>14  (19%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>75</td>
</tr>
<tr>
<td>True (typical) SCID plus Leaky SCID</td>
<td>6</td>
</tr>
<tr>
<td>TCL (idiopathic, secondary, syndromes)</td>
<td>48</td>
</tr>
<tr>
<td>Preterm</td>
<td>3</td>
</tr>
<tr>
<td>False Positive (CD3 ≥ 2500)</td>
<td>18</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>250</td>
</tr>
</tbody>
</table>
PPV of TREC Screen based on number of referrals

• Positive Predictive Value for SCID/Leaky SCID based on 1\textsuperscript{st} NBS
  • 14.3\% of referrals positive 1\textsuperscript{st} NBS (TREC ≤ 60) had either SCID or Leaky SCID
    • 5 patients w/ SCID all had 1\textsuperscript{st} TREC = 0 → 11.9\%
    • 1 patient w/ leaky SCID had 1\textsuperscript{st} TREC = 27 → 2.4\%
PPV of TREC Screen based on number of referrals

• Positive Predictive Value for SCID/Leaky SCID based on 1st NBS
  • 14.3% of referrals positive 1st NBS (TREC ≤ 60) had either SCID or Leaky SCID
    • 5 patients w/ SCID all had 1st TREC = 0 → 11.9%
    • 1 patient w/ leaky SCID had 1st TREC = 27 → 2.4%

• Positive Predictive Value for TCL, CD3 <2500
  • 1st NBS: 61.9%
  • 2nd NBS: 63.2%
## Washington State NBS: SCID Prevalence Rates

<table>
<thead>
<tr>
<th>Year</th>
<th>Infants Screened</th>
<th>True SCID &amp; Leaky SCID prevalence</th>
<th>Other TCL prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>87,398</td>
<td>1:87,398</td>
<td>1:9,710</td>
</tr>
<tr>
<td>2015</td>
<td>88,367</td>
<td>1:88,367</td>
<td>1:6,311</td>
</tr>
<tr>
<td>2016</td>
<td>90,530</td>
<td>0:90,530</td>
<td>1:12,932</td>
</tr>
<tr>
<td>2017</td>
<td>87,815</td>
<td>1:29,271</td>
<td>1:4,181</td>
</tr>
<tr>
<td>TOTAL</td>
<td>354,110</td>
<td>1:59,018</td>
<td>1:6,943</td>
</tr>
</tbody>
</table>
Abnormal/Positive Screens: Not Referred

• 160 total newborns with abnormal/borderline TREC not referred
  • 72 low birth weight (<2500g), gestational age not reported
    • 83% of these infants had normal repeat TREC, 17% expired before repeat
Abnormal/Positive Screens: Not Referred

• 160 total newborns with abnormal/borderline TREC not referred
  • 72 low birth weight (<2500g), gestational age not reported
    • 83% of these infants had normal repeat TREC, 17% expired before repeat
  • 54 normal birth weight (≥ 2500g)
    • 49% had borderline TREC 1 (61-80) with normal repeat
Abnormal/Positive Screens: Not Referred

- 160 total newborns with abnormal/borderline TREC not referred
  - 72 low birth weight (<2500g), gestational age not reported
    - 83% of these infants had normal repeat TREC, 17% expired before repeat
  - 54 normal birth weight (≥ 2500g)
    - 49% had borderline TREC 1 (61-80) with normal repeat

- Subsequent TREC (2nd-4th) which are normal can be reassuring and decrease the need for flow cytometry, thereby also minimize false positive referrals
Conclusions

- Second NBS improves detection of non-SCID TCL → lead to delaying administration of live viral vaccines and/or starting PJP prophylaxis
- Second NBS is helpful in resolving borderline TREC in all newborns, including LBW babies
Conclusions

- Second NBS improves detection of non-SCID TCL → lead to delaying administration of live viral vaccines and/or starting PJP prophylaxis
- Second NBS is helpful in resolving borderline TREC in all newborns, including LBW babies
- First NBS is critical, facilitates timely referral, flow cytometry for patients with SCID, and leaky SCID
  - Interventions include hospitalization, cessation of breastfeeding, prophylaxis to prevent opportunistic infection, HSCT
Conclusions

- Second NBS improves detection of non-SCID TCL → lead to delaying administration of live viral vaccines and/or starting PJP prophylaxis
- Second NBS is helpful in resolving borderline TREC in all newborns, including LBW babies
- First NBS is critical, facilitates timely referral, flow cytometry for patients with SCID, and leaky SCID
  - Interventions include hospitalization, cessation of breastfeeding, prophylaxis to prevent opportunistic infection, HSCT
- Persistently low TREC on 1st and 2nd NBS improves PPV for non-SCID TCL