Newborn Screening for SCID and T Cell Lymphopenia

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Screening Basics

1. Applied to whole population

2. **Not diagnostic.** Identifies a small subpopulation for further testing.

3. Screening tests for rare disorders
   - Inexpensive, high throughput, sensitive.
   - Further testing required to establish diagnosis.
   - Specificity determines the workload for the follow-up testing.

4. Challenges
   - NICU population has higher false positives.
   - Differential diagnosis—workup algorithm.
   - Time to resolution: Diagnose true positives, rule out false positives—minimize “Patients in Waiting.”
   - Clinical significance (“Now that we know the diagnosis, what does it mean for my child?”)
Why Screen Populations for Immune System Disorders?

• Diagnose & treat serious illness early -- improve outcomes.
• Improve public health FOR ALL.
• Learn incidence and spectrum of inherited immune disorders.
• Find new disease genes and mechanisms.
• Use new knowledge to develop new drugs and treatments for rare and more common diseases.
TREC Screening **Primary** Target: SCID

“Classical” SCID

- Failure to thrive, thrush
- Recurrent, severe, & opportunistic infections
- High mortality by age 1
- Absent or low T cells
- B cells absent or non-functional
- Survival requires bone marrow transplant, or enzyme or gene therapy

Improved survival with diagnosis at birth (affected relative)
New Definitions for “Typical” and “Leaky” SCID Based on Lab Criteria

- **Typical SCID**: Absent or impaired T cell production; no functional B cells.

- Fewer than 300/uL autologous T cells; if more T cells are present they may be of maternal origin.

- Absent or very low naïve T cells, poor diversity.

- Abnormal function: less than 10% of lower limit of normal proliferation to PHA.

- Most often with pathogenic mutation(s) in known SCID gene.

- **Leaky SCID**: 300-1500 T cells/uL, functional impairment, few naïve cells, oligoclonality, no maternal engraftment, most often with hypomorphomorphic mutation(s) in known SCID gene.
Thymus produces a diverse T cell repertoire

- Excised DNA forms **T Cell Receptor Excision Circles (TRECs)** as a byproduct.
- TRECs are stable and can be detected by PCR.
- Newborns have the most TRECs; TRECs are diluted as T cells undergo many divisions in the periphery.

**Vα Vδ Rec Dδ Jδ Cδ ΨJα Jα Cα**

**TCRA locus**

**TCRD locus**

**sjTREC**

70% of αβT cells make this

PCR across joint
DNA amplification failure; new sample is needed.
SCID Actual Guthrie Card

Copy Number per Punch (~3ul)

Sample Number

○ TRECs
× Actin
SCID Newborn Screening, May 2015

- Screening by 2012
- Screening 2013-2015
- Implementation awards, DHHS-APHL
- Approved, not funded 2015
- Not screening

29 states plus DC, Navajo Nation, >75% of births
Initial TREC assay, copies/uL of blood

TREC >22

Normal

TREC ≤22

Repeat TREC with β-Actin

TREC >22

β-Actin OK

Positive / Abnormal

β-Actin bad

DAF

β-Actin OK

Regular

NICU

Incomplete

β-Actin bad

DAF

Normal

TREC >22

Low TREC x2

Normal

TREC ≤22

Repeat Heel Stick

DAF or Incomplete

T lymphopenia: <1,500 T cells or absent naïve CD4/CD45RA T cells – Refer to Immunodeficiency Center

Normal: ≥1,500 CD3 T cells with naïve cells present

CBC & lymphocyte subsets are run at one contract Lab & interpreted by NBS Program Immunology Consultants
T lymphopenia: <1,500 T cells or absent naïve CD4/CD45RA T cells – Refer to Immunodeficiency Center

Normal: ≥1,500 CD3 T cells with naïve cells present

Initial TREC assay, copies/uL of blood

TREC >22

TREC ≤22

Repeat TREC with β-Actin

TREC >22

β-Actin OK

β-Actin bad

TREC ≤5

β-Actin OK

β-Actin bad

TREC 6-22

β-Actin bad

Positive / Abnormal

CBC & lymphocyte subsets are run at one contract Lab & interpreted by NBS Program Immunology Consultants
<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
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<tbody>
<tr>
<td>WBC</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td>RBC</td>
<td>2.83 ± 0.8</td>
</tr>
<tr>
<td>HGB</td>
<td>9.7 ± 1.5</td>
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<tr>
<td>HCT</td>
<td>28.6 ± 5.0</td>
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<tr>
<td>MCV</td>
<td>101.0 ± 20.0</td>
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<tr>
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<td>34.3 ± 5.0</td>
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<tr>
<td>MCHC</td>
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<tr>
<td>PLT</td>
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<tr>
<td>MPV</td>
<td>7.9 ± 2.5</td>
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<tr>
<td>RDW</td>
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<tr>
<td>Absolute Neutrophils</td>
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<td>Absolute Band Neutrophils</td>
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</tr>
<tr>
<td>Absolute Promyelocytes</td>
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<tr>
<td>Absolute Lymphocytes</td>
<td>630 ± 2500</td>
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<tr>
<td>CD3 T-Cells, Absolute</td>
<td>&lt;20 ± 10</td>
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<tr>
<td>CD3 T-Cells, Percent</td>
<td>&lt;1 ± 0.1</td>
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<tr>
<td>CD4 T-Helper, Absolute</td>
<td>&lt;20 ± 10</td>
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<tr>
<td>CD4 T-Helper, Percent</td>
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<tr>
<td>CD8 T-Cytotoxic, Absolute</td>
<td>&lt;20 ± 10</td>
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<tr>
<td>CD8 T-Cytotoxic, Percent</td>
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<tr>
<td>CD19 B-Cells, Absolute</td>
<td>328 ± 52</td>
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<tr>
<td>CD19 B-Cells, Percent</td>
<td>12 ± 6</td>
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<tr>
<td>CD16/56 NK-Cell, Absolute</td>
<td>132 ± 17</td>
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<tr>
<td>CD16/56 NK-Cell, Percent</td>
<td>21 ± 4</td>
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<tr>
<td>CD3/CD4/CD45RA, Absolute</td>
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<tr>
<td>CD3/CD4/CD45RA, Percent</td>
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<tr>
<td>CD3/CD8/CD45RA, Absolute</td>
<td>&lt;20 ± 10</td>
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<tr>
<td>CD3/CD8/CD45RA, Percent</td>
<td>&lt;1 ± 0.1</td>
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</table>
4 Years of California SCID Newborn Screening (2010-2014)

1,980,133 infants screened

109/255 had <1500 T cells/μL (43%)

1/55,000 SCID (Typical and Leaky)

1/180,000 idiopathic TCL

Typical SCID 12%

Syndrome 13%

Secondary 5%

Preterm 6%

Leaky SCID 3%

Idiopathic TCL/Variant SCID 4%

Normal T cells by flow 57%

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Genotypes of Typical and Leaky SCID

Reports from Transplant Centers, no Screening
*Duke University, European centers (estimates)*

- IL2RG 50%
- RAG1 1%
- ADA 14%
- IL7R 10%
- JAK3 7%
- DCLRE1C 5%
- RMRP 1%
- TTC7A 1%
- Unknown 3%

Overall Survival ~74% or lower

California, with TREC Screening
*4 years, ~2 million infants*

- IL2RG 28%
- RAG1 18%
- ADA 12%
- IL7R 15%
- RMRP 3%
- RAG2 6%
- JAK3 6%
- Unknown 12%

Overall Survival 95%
T Cell Number and Diversity: Vβ TCR Spectratyping in a SCID patient

At diagnosis: few T cells, mono- or oligoclonal peaks

1 yr post-HCT: Normal pattern with many peaks, normal size distribution

3 mo post-HCT
PIDTC: Active infection, age at HCT affect 5 year survival in *Typical SCID*

Comparison of all groups to >3.5m with active infection are significant. Comparison of >3.5m no infection or >3.5m infection resolved to <3.5m are NS.

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6% Preterm

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12% Typical SCID

4% Idiopathic TCL/Variant SCID
Known Syndromes with Variable T Cell Defects (can be profound)

- DiGeorge syndrome
- Trisomy 21
- Ataxia telangiectasia
- Nijmegen breakage syndrome
- CHARGE syndrome
- Cartilage hair hypoplasia
- Jacobsen syndrome
- RAC2 deficiency, dominant interfering mutation
- DOCK8 deficiency
- CLOVES syndrome
- Fryns syndrome (diaphragmatic hernia, anomalies)

Others…
Secondary T Lymphopenia

- Congenital heart disease (heart surgery with thymectomy)
- Gastroschisis, gastrointestinal atresia
- Vascular leakage, chylothorax, third spacing
- Neonatal leukemia
- Extreme prematurity
- Maternal immunosuppressive medication
- *Prenatal HIV infection (hypothesized, not yet found)*

  T cells normalize upon resolution of the primary pathogenic process
Preterm Low Birthweight Infants with Low TREC and T Lymphopenia

CD3 T cells /uL vs Age in weeks

Threshold for follow-up

BW 300g
BW 490g
BW 560g
BW 445g
BW 700g
BW 557g
Idiopathic T Lymphopenia (Variant SCID)

- Persistent low but not absent T cells and TREC, low naïve CD45RA T cells, no maternal engraftment.
- No known SCID gene mutation.
- Impaired T cell and/or antibody responses.
- When an etiology is found, the case is moved to the appropriate category.
Opportunities to Learn Population Based Aspects of T cell Immune Deficiency

- SCID, 1/54,000 births
- All T lymphopenia <1500/uL 1/23,000 births
- DiGeorge, 22q11.2 deletion
  - Overall incidence ~1/5,000 births (literature).
  - Broad spectrum of phenotypes, including variable T cell immunodeficiency.
  - California cases with <1,500 T cells/uL represent 5% of all cases
Following Infants with Low Lymphocytes

FILL Project

- **Aim**: explore spectrum of disorders
  - Enroll infants with T cell lymphopenia early in life of any known/unknown cause
  - Collect data at 3 time points: 3 mo, 6-9 mo, 12-18 mo
  - Form basis for future studies
  - Hope to enroll 200 cases in first year

- **Support**: CIS, USIDNET, Modell Foundation
- **Data fields** programmed into USIDNET Registry
- **Patient consent** online or through USIDNET Centers
- **Rewards** for physicians who enter data
TREC Screen Does Not Pick Up All CID or PID

- Defect after TCR recombination in thymus; T cell number or diversity not reduced
  - CD40L deficiency, Hyper-IgM syndrome
  - MHC II deficiency
  - ZAP70 deficiency
- SCID gene defect sufficiently leaky to allow TRECs to be normal (late onset ADA deficiency)
- Syndrome with variable T cell deficiency with enough T cells to have TRECs above cutoff
- PID not involving T cells
  - XLA, CGD, etc.
- PID not evident at birth
  - CVID
Conclusions

1. SCID is the most serious treatable genetic immune deficiency, affecting around 1/50,000 births.

2. Early diagnosis permits optimal treatment and outcomes.

3. Population based newborn screening with TRECds identifies SCID, and also non-SCID conditions with low T cells, offering clinical benefit and opportunities to define the spectrum of disorders.

4. A high index of suspicion is still needed for primary immune defects not picked up by TREC screening.
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PIDTC U54 Primary Immune Deficiency Treatment Consortium
NCATS: UCSF CTSI

IDF Immune Deficiency Foundation
JMF Jeffrey Modell Foundation
CDC Center for Disease Control and Prevention
DHHS Maternal & Child Health Bureau, NBSTRN