NEWBORN SCREENING FOR SCID: THE SCIENCE BEHIND TREC ANALYSIS AND IMPLEMENTATION IN SOUTH CAROLINA

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Roadmap

- Case report
- T Cells and their importance
- SCID: Clinical features, diagnosis, and management
- TRECs and development of NBS for SCID
- The Wisconsin Experience
- Implementation in SC
- Q&A


- Four month old WF is admitted to your pediatric ward with the following:
  - Recurrent febrile illnesses
  - Oral ulcer
  - Failure to thrive

- Pmhx:
  - TVD, no complications
  - GERD evaluation by GI at 2 months of age
  - Otitis Media 2 months – treated by amoxicillin 10 days
  - Otitis Media 3 months – treated by Augmentin 10 days
  - Chronic cough and nasal drainage
Case

• Family history:
  • Healthy 2 year old brother with recurrent OM, now resolved at PET
  • Twin sisters born premature and died shortly after birth
  • No consanguinity

  • Px: 36.5 C 110 35 103/53 88% on room air
  • Wgt 5.5 kg (10% for age)
  • Hgt 62.5 cm (25% for age)
  • Gm: coughing infant
  • Oral: 2x3 cm with yellow ulcer pseudomembranous covering, no tonsils
  • Pulm: bilateral wheezing
  • Cv: HR, no murmur
  • Abd: liver edge palpated 4 cm below right costal margin
  • Extr: no rash, no c/c/e, no peripheral lymph nodes palpated

• Labs and Imaging
  • WBC 1500/mcl
  • H/H/Platelets NML
  • ANC 795/mcl (low)
  • ALC 165/mcl (low)
  • AST 1144 (35-140 U/L)
  • ALT 385 (5-50 U/L)
  • Nasal viral cx + para-influenza 3
  • Blood Adenovirus PCR positive
  • CMV negative, HIV negative

• Diagnosis - SCID
  • IgG 65 mg/dl (192–515)
  • IgA 0 mg/dl (12–31)
  • IgM 0 mg/dl (39–92)
  • IgE 5 IU/ml (0–150)
  • Anti-A, titer 0 >1:32
  • Anti-B, titer 0 >1:16
  • Diphtheria toxoid antibody 0.19 IU/ml (>0.10)
  • Tetanus toxoid antibody 0.05 IU/ml (>0.16)
  • Lymphocyte subpopulations
    • CD3 T cells 1 (1111–5183)
    • CD20 B cells 0 (144–671)
    • CD16 NK cells 142 (152–709)
    • CD56 NK cells 134 (223–1040)
  • Proliferation study
    • Phytohemagglutinin 150 (184 710 +/- 59 291)
Patient Case - SCID
- RAG2 mutation identified by sequencing (AR mutation).
- Patient was started on bactrim and IVIG and referred for bone marrow transplant.
- On fifth day of hospitalization, patient developed a fever 40°C, diffuse petechial rash, oozing from multiple venipuncture sites, and was diagnosed with DIC.
- Ultimately, the patient passed away from liver failure secondary to adenovirus infection.
- Clinical Pearls:
  - CBC not drawn until 6 weeks after mouth ulcer diagnosed.
  - Low ALC on outpatient not appreciated.
  - Lack of thymic shadow not reported on admission CXR.

Severe Combined Immunodeficiency
- SCID is a primary immunodeficiency that can be caused by 13 different mutations, that all result in T cell lymphopenia and variable deficits in B and NK cell populations.
- Medical Emergency.
- X-linked SCID (IL-2R gamma chain mutation) comprises roughly 40% of all diagnoses
- Patients commonly present within 3 months of life with severe disseminated viral, bacterial, or fungal infections.
- Failure to thrive and rashes also common.
- Universally fatal under 2 years of age if not treated.

T Cells
T Cells

- Lymphoid progenitors leave bone marrow in utero and early infancy and arrive in thymus for T cell development and "education."
- T cells undergo positive and negative selection processes in thymus, allowing them to become T helper cells or cytotoxic T cells.
- Thymic emigrants are mature, but naïve T cells.
- T cells comprise roughly 70% of all lymphocytes in newborns.

T Cell Quantity By Age

<table>
<thead>
<tr>
<th>Age</th>
<th>CD 3+ cells x 10^9 /L (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal</td>
<td>0.6-6.0 (28-76)</td>
</tr>
<tr>
<td>1 week – 2 months</td>
<td>2.3-7.0 (60-85)</td>
</tr>
<tr>
<td>9 – 15 months</td>
<td>1.6-6.7 (54-76)</td>
</tr>
<tr>
<td>2 – 5 years</td>
<td>0.9-4.5 (43-76)</td>
</tr>
<tr>
<td>5 – 10 years</td>
<td>0.7-4.2 (55-78)</td>
</tr>
</tbody>
</table>

SCID: Clinical Features

- True incidence unknown (1:40K).
- Family history of immunodeficiency uncommonly reported.
- Delay in diagnosis common (> 6 months of age).

**Physical Exam:**
- Underweight
- Absence of lymphoid tissue
- Vestigial thymus
- Erythroderma
- Evidence of fungal or bacterial disease (thrush, soft tissue infections)

**Laboratory:**
- Low to absent immunoglobulins (IgG, IgA, IgM).
- T cell lymphopenia
- B and NK cell deficits variable
- Severe reduction of T cell proliferation to antigens and mitogens
- Leukopenia

**Diagnosis:**
- Genetic Analysis

**Management:**
- Avoidance of live viral vaccines
- Infection precautions
- IVIG
- Leukopoor irradiated CMV negative blood transfusion
- Breast-feeding?
- Acyclovir and Bactrim prophylaxis
- Palivizumab
- HSCT!!!!
### Mutation T cell B Cell NK cells

<table>
<thead>
<tr>
<th>Mutation</th>
<th>T cell</th>
<th>B Cell</th>
<th>NK cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2RG (X-linked)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ADA</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>IL7R</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>JAK3</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>RAG 1 or 2</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>TCR (D,E,Z)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD45</td>
<td>-</td>
<td>+</td>
<td>+/Low</td>
</tr>
<tr>
<td>LCK</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PNP</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>IGH</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DNA PKCS</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>NHEJ</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CORO1A</td>
<td>-/Low</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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### HSCT for SCID

**Effect of age at the time of transplantation on survival in 169 SCID patients undergoing transplantation at Duke University Medical Center from 1982-2011.** 49 of the infants were < 3.5 months of age when they underwent nonchemoablated transplantation. All but 3 (94%) of the 49 infants are surviving.


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### Sources of stem cells for transplantation

- HLA-identical sibling donor
- Haploidentical parental donor
  - T-cell depleted by soy lectin/SRBC
  - T-cell depleted by mAbs*
- Matched unrelated adult donor*
  - Unfractionated*
  - T-cell depleted*
- Unfractionated related or unrelated cord blood*

*In most protocols pre-transplantation chemotherapy and post-transplantation GVHD immunosuppressive drugs are given.
*Requires time for a registry search
Summary of Wilson and Jungner criteria for Newborn Screening

- Prevalence of disease warrants cost of NBS.
- Disorder not readily identified by means of physical examination.
- Disease must cause serious medical complications.
- Early diagnosis and treatment of disease improves prognosis.

- Acceptable, sensitive, specific, economic, and proved screening test must be available (this is where TRECs come in......)
Development of TREC Detection

- In 2005, Chan and Puck published a study showing that real time quantitative PCR could determine TREC count in dried blood spots collected on Guthrie cards from newborns.
- Healthy control samples were provided from the Maryland Newborn Screening program and TREC counts quantified from these samples were compared directly with Guthrie samples of known SCID cases.
- Average TREC count for healthy controls were 1020 over two 3 mm dried blood spot punches versus < 30 for known SCID cases.
TRECs < 25/mcl?
Confirmation testing should include flow cytometry to evaluate the following:
- CD 3+
- CD 4+ CD 45 RA/RO
- CD 8+
- CD 19+
- CD 16+
- CD 56+

Wisconsin 2007
- Clinical immunologists at the Children’s Hospital of WI receive IRB approval for a proof of concept study.
  - De-identified 5,766 dried blood spots for TREC testing.
  - TREC number/3 mcl was 0 – 3900.
  - Mean and median values were 827 and 708 respectively.
  - 1.06% of sample had TREC number of < 25/mcl.
Wisconsin NBS SCID 2008 (n= 71K)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Term Infants</td>
<td>64,397</td>
<td>90.7</td>
</tr>
<tr>
<td>Inconclusive results (repeat specimen)</td>
<td>23</td>
<td>0.03</td>
</tr>
<tr>
<td>Abnormal results (referral for flow cytometry)</td>
<td>12</td>
<td>0.02</td>
</tr>
</tbody>
</table>

As of 2012, Wisconsin has identified 4 cases of SCID and 7 cases of T cell Lymphopenia out of 243,707 newborns. All received either HSCT or enzyme replacement therapy and are surviving.

Baker MW et al. Implementing testing for SCID within Wisconsin’s NBS. PHL. 2010 (2); 125: 88-95.

Pearls from Wisconsin Experience

- **Funding**
  - Development of pilot and start up costs: $630K
  - Cost to screen infants for first year: $420K
  - Estimated cost of one test after first year of screening: $6.00

- **Testing Efficacy**
  - False positive rate <1%
  - SCID NBS did not delay testing of diseases on NBS panel.
  - 0.17% results considered inconclusive.
  - Intra-laboratory repeat punch testing cleared 99.88% of all full term infants.
NBS for SCID

- January 2010: Department of Health and Human Services receives recommendations that the federal government recommend the inclusion of SCID into newborn screening protocols.
- May 2010: Secretary Sebelius concurs with an advisory committee’s recommendations to incorporate SCID into a core panel of newborn screening.
- States currently screening for SCID include: WI, MA, NY, CA, CT, MI, CO, MS, DE, FL, TX, MN, IA, PA, UT, OH, and WY, Navajo nation.

TREC Results from Other States

<table>
<thead>
<tr>
<th>State</th>
<th>Start Date</th>
<th>Yearly Birth #</th>
<th>SCID</th>
<th>SCID Variant</th>
<th>Non SCID</th>
</tr>
</thead>
<tbody>
<tr>
<td>WI</td>
<td>1/1/08</td>
<td>69,232</td>
<td>4</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>MA</td>
<td>2/1/09</td>
<td>77,022</td>
<td>1</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Navajo</td>
<td>2/1/09</td>
<td>2,000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NY</td>
<td>9/10/10</td>
<td>236,656</td>
<td>4</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>CA</td>
<td>8/1/10</td>
<td>510,000</td>
<td>5</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>PuertoRico</td>
<td>8/1/10</td>
<td>45,620</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>LA</td>
<td>10/1/10</td>
<td>66,268</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>n/a</td>
<td>n/a</td>
<td>14</td>
<td>6</td>
<td>40</td>
</tr>
</tbody>
</table>
Diagnoses with low T cells (< 1500 cells/mcL)

- Typical SCID
  - < 300 autologous T cells/mcL
  - < 10% normal mitogen proliferation to PHA
- Leaky SCID
  - Hypomorphic mutation in a typical SCID gene
  - 300-1500 T cells/mcL
  - 10-30% normal mitogen proliferation to PHA
- Variant SCID
  - No identifiable mutation
  - 300-1500 T cells/mcL
  - Impaired function

- Syndromes with Variable Cellular ID
  - DiGeorge Syndrome
  - Jacobsen Syndrome
  - CHARGE
  - Trisomy 21
  - DOCK8 Hyper-IgE Syndrome
  - Rac2 Dominant Interfering Mutation
  - Cartilage Hair Hypoplasia

- Secondary TCL
  - Neonatal Leukemia
  - Gastrochisis
  - Third Spacing
  - Neonatal Cardiac Surgery with Thymectomy
  - Severe Prematurity
  - Severe HIV Perinatal Disease
NBS for SCID in SC

- DHEC panel of Pediatricians, Immunologists, Geneticists, Neonatologists, ID Specialists, and Hematologist-Oncologists convened for implementation of NBS for SCID in October of 2013.
- Patients with significant T cell lymphopenia would be considered candidates for possible HSCT at MUSC Children’s Hospital.
- Funding issues
- Training
- Awareness

Newborn in SC with low TREC's?

- Regional expert and local primary doctor notified.
- Verification of specimen quality
- Flow cytometry to evaluate for T cell quantity at regional children’s hospital lab: GHS, McLeod, Palmetto Health Children’s, and MUSC Children’s.
- Normal result
- T cell lymphopenia -> Immunology referral for immediate treatment and possible evaluation of HSCT.
Pitfalls and Barriers

- Neonatal Screening
- Severe prematurity frequently associated with low TREC levels
- Secondary conditions associated with low TREC levels are common diagnoses in NICUs
- Prenatal steroid use and its effect on neonatal immune system
- Lack of age matched controls for ALC and CD4+ cells in premature neonates
- When compared with full term controls, TREC numbers in premature neonates increase weekly by 9.8%.
- Cost of Flow Cytometry for Confirmation
- Awareness of Algorithm and Implementation of Protocol
- Limitations of TREC test (what it doesn’t do,...)

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