SCID/DiGeorge Syndrome: TREC's Update

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Disclosure

• I have no relevant financial relationships with the manufacturers(s) of any commercial products(s) and/or provider of commercial services discussed in this CME activity

• I do not intend to discuss an unapproved/investigative use of a commercial product/device in my presentation.
Objectives

- Describe severe combined immune deficiency (SCID)
- Describe the variety of abnormalities that can lead to a T cell deficiency
- Explain the diagnostic tests that might be used to further evaluate a patient for T cell immune deficiencies
- Describe the clinical presentation of DiGeorge Syndrome
- Describe T Cell Receptor Excision Circle (TREC) testing in newborns
- Identify other diseases that may be diagnosed with TREC newborn screening
- Describe the standard management for a newborn with a positive newborn screen

Primary Immune Deficiency

- Immune deficiency not caused by other factors (secondary immune deficiency)
  - Disease
  - Medications
  - Malnutrition
- Estimated 200 primary immune deficiency diseases
- 1:1,200 live births have an immune deficiency
Primary Immune Deficiency

- Humoral (antibody, B lymphocytes affected)
  - Selective IgA deficiency or common variable immune deficiency
- Cellular (T lymphocytes affected)
  - DiGeorge Syndrome
- Combined (T, and/or B, and/or NK cells affected)
  - SCID subtypes
- Phagocytic
  - Chronic granulomatous disease
- Primary ciliary dyskinesia

Case 1

- HPI:
  - Female born via C section at 38 weeks and 5 days due to failure of progression of labor.
  - Infant was born without adequate respiratory effort. However, by 4-5 minutes, baby improved.
  - Patient transferred to the neonatal ICU for further observation.
Case 1

• Family History
  – Mother: healthy
  – Father: healthy
  – Paternal aunt has severe combined immune deficiency
  – Maternal 1st cousin and paternal uncle died in infancy
  – 2 paternal 1st cousins with primary ciliary dyskinesia

• Social history
  – Significant for being Irish travelers
  – High incidence of consanguinity

Case 1

• Physical Exam
  – Vitals: RR 29, SpO2 100%, HR 108, BP 56/35, Temp 36.7°C
  – General: no acute distress
  – HEENT: normocephalic, fontanelles were normal in size, normal eyes without conjunctival erythema or drainage, well positioned ears and normal pinnae, patent nares with normal mucosa, oropharynx is clear, palate was intact
  – Respiratory: clear to auscultation, unlabored breathing
  – Cardiovascular: regular rate and rhythm, normal S1, S2, no murmurs or gallops
Case 1

• Physical Exam:
  – GI: normal appearance, soft, non-tender, without organ enlargement or masses
  – GU: normal female external genitalia
  – Neurologic: moves all extremities, good tone
  – Spine: spine intact
  – Extremities: normal
  – Skin: pink without rash

<table>
<thead>
<tr>
<th>Lab</th>
<th>Level</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>16.3</td>
<td>Low</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>48.6</td>
<td>Low</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>313</td>
<td>Normal</td>
</tr>
<tr>
<td>White Blood Cells</td>
<td>14.4</td>
<td>High</td>
</tr>
<tr>
<td>Segmented Neutrophils (%/Abs)</td>
<td>63%/11.3</td>
<td>Normal</td>
</tr>
<tr>
<td>Bands</td>
<td>15%</td>
<td>High</td>
</tr>
<tr>
<td>Lymphocytes (%/Abs)</td>
<td>9%/1296</td>
<td>Low</td>
</tr>
<tr>
<td>Monocytes</td>
<td>12%</td>
<td>Normal</td>
</tr>
<tr>
<td>Eosinophils (%/Abs)</td>
<td>1%/1.4</td>
<td>Normal</td>
</tr>
</tbody>
</table>
Case 1

- IgG 941 (614-1536 mg/dL)
- IgA <10 (0-7 mg/dL)
- IgM <20 (6-23 mg/dL)

### Lymphocyte Subsets Results

<table>
<thead>
<tr>
<th>Lymphocyte Subsets</th>
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<tbody>
<tr>
<td>CD3+ T cells %</td>
<td>0.4% (58-67%)</td>
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<td>CD8+ T cells %</td>
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</tr>
<tr>
<td>CD8 absolute</td>
<td>2 (800-1200)</td>
</tr>
<tr>
<td>CD16/56 NK cells %</td>
<td>95.9 (8-17%)</td>
</tr>
<tr>
<td>CD16/56 absolute</td>
<td>435 (300-700)</td>
</tr>
<tr>
<td>CD19 B cells %</td>
<td>0 (19-31%)</td>
</tr>
<tr>
<td>CD19</td>
<td>0 (500-1500)</td>
</tr>
</tbody>
</table>
Absence of the thymic shadow – lack of the sail sign

Case 1

Thymus shadow is missing

Normal cardiothymic silhouette

Normal CXR
Case 1

• So what is the diagnosis?
• SCID:
  – T-B-NK+
• Genetic evaluation:
  – RAG-1 Defect

Severe Combined Immune Deficiency (SCID)

• Initially described in 1950
• Syndrome caused by mutations in different genes whose products are necessary for T/B/NK cell development
• Leads to early death from overwhelming infection within the first year of life if not transplanted
• 1:40,000-1:50,000 live births
  – 1:2,500 in Navaho population
• 45% are X-linked SCID
Clinical features

• Within the first few months of life:
  – Recurrent episodes of diarrhea, pneumonia, otitis, sepsis and cutaneous infections
  – Failure to thrive (may be normal initially)

• Opportunistic organisms:
  – Candida albicans, Pneumocystis jirovecii, varicella, adenovirus, parainfluenza, herpes viruses, cytomegalovirus, rotavirus, measles, norovirus and Epstein-Barr

Clinical features

• Attenuated vaccine organisms can cause severe or fatal infection
  – Polio, rotavirus, varicella and BCG

• Diagnosis may be delayed several months since infants look normal and maternally-derived antibodies provide some protection in early infancy
  – IgG crosses the placenta
    • Nadir 4-6 months or earlier
  – IgA and IgM may be low and still at age appropriate levels
Clinical features

- Small thymus (less than 1 gm)
  - Fails to descend from neck
  - Few thymocytes
  - Lacks corticomedullary distinction & Hassall’s corpuscles
  - Presence of a thymic shadow does not rule out SCID as it may be present in rare forms

A defect may occur at nearly any area in the T cell receptor activation, cell signaling, or with subsequent gene transcription to cause a T cell deficiency
Severe combined immunodeficiency (SCID) classification and gene defects

| T-B-NK: SCID |  |  |
| X-linked SCID | common gamma chain (gamma-c) | IL2RG |
| Jak3 kinase | Jak3 |
| T-B-NK: SCID |  |  |
| IL-7 receptor alpha chain (CD127) | IL7RA |
| Janus-regulating receptor 1a (JAK1) | JAK1A |
| CD3 gamma chain | CD3G |
| CD2 epsilon | CD2E |
| CD3 delta | CD3D |
| CD45 | CD45 |
| T-B-NK: SCID |  |  |
| Ascorbate activating genes 1 and 2 | AASS, AASS2 |
| Artemis | DNA-PKcs |
| DNA polymerase catalytic subunit (DNA-Pol) | DNA-PKcs |
| DNA ligase IV | LIG4 |
| Complement/C4-like factor (DAF) | C4L2 |
| T-B-NK: SCID |  |  |
| Adenosine deaminase | ADA |
| Adenosine deaminase | ADA |

The defects listed in bold are the most common forms of SCID.
- SCID: X-linked, non-SCID: combined immunodeficiencies.

* Some defects in these genes lead to early, non-SCID combined immunodeficiencies.

- Some patients with X-linked SCID have NK cells present at low levels and would thereby be classified as having X-linked SCID.
SCID Evaluation

- Peripheral lymphocyte count (infant or cord blood)
- TREC (T cell excision circles) analysis
  - Neonatal screening
  - Measures T cells emigrating from the thymus (mature)
  - Low to absent in neonates who make no or few T cells
  - Slightly lower in premature infants
- Lymphocyte subsets (T, B, NK cell enumeration)
- Lymphocyte proliferation (mitogens and antigens)
- Immunoglobulin levels (IgG, IgA, IgM, IgE)
- Specific antibody responses
  - If > 6 months of age

SCID Evaluation

- Strong suspicion for SCID suspicion.
  (or another T cell immune deficiency):
  - Absolute lymphocyte count < 2500 cells/mm3
  - T cells making up less than 20% of the total lymphocytes
  - Lymphocyte stimulation to mitogens is < 10% of the normal control
  - Absence of the thymus on CXR
SCID Evaluation

- Serum immunoglobulin concentrations may be low or absent
  - Even if B cells are present, they do not make immunoglobulins
    - Possibly even after transplantation
  - May have normal IgG levels for the first few months of life
    - Transplacental maternal transfer
- Lack of antibody formation after immunization
- T cells always diminished or absent
- Variable presence/absence of B/NK cells determines the defect

X-linked SCID (T-B+NK-)

- About 45% of infants with SCID
- Defect on X chromosome encoding the cytokine receptor subunit common gamma chain, IL2 R gamma
  - Diagnosed on flow cytometry
  - Receptor subunit shared by six different cytokine receptor complexes: IL-2, 4, 7, 9, 15 and 21
  - Gamma-chain is also involved in growth hormone receptor signaling
    - Growth failure may be partially due to this
Autosomal Recessive SCID

- First described in 1950 in Switzerland
- More common in Europe
- 12 genetic types
  - ADA, JAK3, IL-7 receptor alpha, RAG-1 or RAG-2, Artemis, ligase 4 deficiency, DNA-dependent PKCs (catalytic subunit), CD3 (delta, epsilon or zeta) and CD45 deficiencies
- May present as Omenn Syndrome
  - “Leaky” hypomorphic SCID

ADA deficiency (T-B-NK-)

- 16% of SCID patients
- Accumulation of adenosine, 2’-deoxyadenosine and 2’-O-methyladenosine
  - Latter two metabolites lead directly or indirectly to apoptosis of thymocytes and circulating lymphocytes
- More profound lymphopenia than other SCID types
  - Absolute lymphocyte count < 500/uL
- Treatment: transplant, PEG-ADA
IL-7 receptor alpha chain deficiency (T-B+NK+)

- Third most common SCID phenotype
- IL-7 receptor alpha is specific only for T-cell development
- Can present as Omenn syndrome
- Patients acquire normal B cell function after haploidentical bone marrow stem cell transplantation without donor B cells
RAG-1 and RAG-2 (T-B-NK+)

- Involved with VDR rearrangement of T and B cell antigen receptors
- Can also present with Omenn syndrome
- Fatal unless corrected with transplantation

This is our patient’s diagnosis
Transplantation winter 2015, doing well!
Radiosensitive

- Inability to repair DNA damage with several types;
  - Artemis (T-B-NK+)
  - Ligase IV Deficiency (T-B-NK+)
  - DNA-dependent protein Kinase catalytic subunit deficiency (T-B-NK+)
  - Nijmegen breakage syndrome
  - Cernunnos/XLF deficiency
- Avoid all unnecessary imaging

Omenn Syndrome

- Leaky SCID - Autosomal recessive
  - Hypomorphic missense mutations:
    - RAG1 and RAG2, IL-7 Receptor α gene, Artemis, DNA-Ligase IV, common gamma chain, ZAP-70, DNA dependent PKC deficiency.
  - Abnormal auto-reactive T cells
    - Low T cells and no B cells
    - Symptoms similar to GVHD: rash (erythroderma), eosinophilia, FTT, lymphadenopathy, diarrhea, hepatosplenomegaly, elevated IgE, low IgG/IgM/IgA
Differential Diagnosis

- Extreme malnutrition
- HIV/AIDS
- Cystic fibrosis
- Wiskott-Aldrich (x-linked)
  - WASp gene mutation
- DiGeorge syndrome
- Hyper IgM syndrome

Doctor’s Strike
Case 2

• HPI: 3 day old infant having problems with hypoxia and turning blue, found to have a heart defect on exam and has been placed in the NICU. ECHO is done and it seems the baby has a heart defect. Labs show a low calcium level and has intermittent facial tetany. He is not feeding well.

Case 2

• Physical Exam
  – Vitals: RR 55, SpO2 85%, HR 180, BP 68/35, Temp 36.7°C
  – General: mild acute distress that is worse when crying
  – HEENT: normocephalic, fontanelles were normal in size, normal eyes without conjunctival erythema or drainage, low set ears, patent nares with normal mucosa, short philtrum, oropharynx is clear, cleft palate
  – Respiratory: clear, tachypnea
  – Cardiovascular: tachycardia, +murmurs
Case 2

• Physical Exam:
  – GI: normal appearance, soft, non-tender, without organ enlargement or masses
  – GU: normal male external genitalia
  – Neurologic: moves all extremities with normal tone, has some intermittent spasms noted
  – Spine: spine intact
  – Extremities: normal
  – Skin: pink without rash

• FH:
  – Mother and father are healthy
  – No siblings
  – No other early childhood deaths in the extended family, no immune abnormalities

• SH:
  – No consanguinity
  – Family is of unknown descent
**Case 2**

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Absence of the thymic shadow  
– lack of the sail sign
Case 2

• So what is the diagnosis?
  – T-/B+/NK+ SCID?
• DiGeorge Syndrome
  – 22q11.2 deletion
  – Defect in the development of the pharyngeal pouch system

DiGeorge Syndrome

• Clinical presentation (CATCH 22)
  – C – Cardiac anomalies
  – A – Abnormal facies
  – T – Thymus aplasia
    • Hypoplasia in partial DiGeorge
  – C – Cleft palate
  – H – Hypocalcemia/Hypoparathyroidism
  – 22 – 22q11.2 deletion
Clinical Non-Immune Findings

- Palate abnormalities
- Cardiac abnormalities
- Developmental delay
  - Speech delay
  - Learning disabilities
- Ophthalmologic abnormalities
- Hypocalcemia
- Psychiatric disorders
- Skeletal abnormalities
- Renal, neurologic, dental abnormalities
- Short stature

DiGeorge Syndrome

- Complete DGS is a form of SCID
  - Found in <1% of patients with 22q deletion syndromes
  - Thymus is absent
  - T lymphocytes are absent
  - Fatal unless treated promptly with thymic or bone marrow transplant
- Diagnosis
  - Newborn screening
  - Lymphocyte subsets (T, B, NK cells)
Phenotypic Findings

• Ocular hypertelorism
• Upslanting palpebral fissures
• Low set posteriorly rotated ears
• Widened area below nasal bridge
• Hooded eyelids
• Bulbous nose tip
• Micrognathia
• Short philtrum
• High arched palate
• Cleft palate / bifid uvula
• Tapered fingers

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Diagnostic criteria for DiGeorge syndrome

<table>
<thead>
<tr>
<th>Definitive diagnosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male or female patient with reduced numbers of CD3+ T cells (less than 500/mm³) and two of the three following characteristics:</td>
</tr>
<tr>
<td>1. Congenital cardiac defect (transposition of great vessels, interrupted aortic arch, or aberrant right subclavian).</td>
</tr>
<tr>
<td>2. Hypocalcemia of greater than three weeks' duration that requires therapy.</td>
</tr>
<tr>
<td>3. Deletion of chromosome 22q11.2.</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Probable diagnosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male or female patient with reduced numbers of CD3+ T cells (less than 1000/mm³) and a deletion of chromosome 22q11.2.</td>
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</table>

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<thead>
<tr>
<th>Possible diagnosis*</th>
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</thead>
<tbody>
<tr>
<td>Male or female patient with reduced numbers of CD3+ T cells (less than 1500/mm³) and at least one of the following:</td>
</tr>
<tr>
<td>1. Cardiac defect.</td>
</tr>
<tr>
<td>2. Hypocalcemia of greater than three weeks' duration that requires therapy.</td>
</tr>
<tr>
<td>3. Dysmorphic faces or palatal abnormalities.</td>
</tr>
</tbody>
</table>

* Patients with a definitive or probable diagnosis are assumed to have a greater than 68 and 89 percent probability, respectively, that in 20 years they will still have the same diagnosis. Patients with a possible diagnosis are those that have some but not all of the characteristic clinical or laboratory findings of a particular disorder.

Summary T Cell Deficiencies

**SCID**
- Defect in T cell production
- +/- NK and/or B cell #s
- Opportunistic organisms
- Imaging: small or absent sail sign (small thymus)
- Absent immunoglobulins
- Diagnosis: TRECs analysis, total lymphocyte count, genetics
- Treatment:
  - HSCT, IVIg, antimicrobials

**DiGeorge Syndrome**
- Defect in T cell production
- Normal NK and B cell #s
- Opportunistic organisms
- Imaging: absent sail sign in complete
- Absent/low immunoglobulins
- Diagnosis: TRECs analysis, genetics
- Treatment: thymus or HSCT, IVIg

**T Cell Receptor Excision Circles (TRECs)**
- TRECs analysis newborn screening
  - Detect T cell defects with screening
  - Sensitive, specific
  - Low false positive rate
  - Cost effective
TRECs Analysis

VDJ Recombination yields excision circles as an activated T cell receptor is formed.

These excision circles are discarded in the form of TRECs.

TRECs analysis

- Severe Combined Immune Deficiency
- Omenn's Syndrome (Leaky SCID)
- DiGeorge Syndrome
- Ataxia Telangiectasia
- Trisomy 21
- Pre-term infants
- T cell lymphopenia secondary to other systemic disorders
  - Cardiac abnormalities
  - Gastrointestinal abnormalities
TRECs Cost Savings

• Cost vs benefit ratio – $4.36
  – $4 saved for every $1 spent
• Treatment costs
  – $350,000 per baby savings in early vs late treatment
• Life value – $7.9 million
• $2.8 million saved per year if 1 baby is saved every 3 years
• Pilot in Kansas 2017 – Go-live 2018

Treatment

• **Pediatric emergency**
• Hospitalization in most cases for isolation
• Antimicrobial prophylaxis
• Intravenous immunoglobulin
• HLA-identical or haploidentical donor bone marrow transplantation needed for survival
  – Prior to 3.5 months of life: 95% chance of survival at 20 years
  – After 3.5 months of life: <70% chance of survival
Positive TREC Screening

- Contact the family
  - Inform them of the results, provide information
  - Start additional testing
  - Alert mom to stop breast feeding until CMV status can be assessed
  - Avoid any exposure to live viral vaccines
- Any evidence of infection
  - Refer to Children’s Mercy Hospital or the University of Kansas Medical Center emergently
- Blood products needed
  - Ensure they are leuko-reduced, irradiated, and CMV negative

SCID

- Positive screen = further testing
  - Repeat if premature/low birth weight
  - Complete blood count
  - Lymphocyte subsets (T, B, and NK cell panel)
  - Quest stat labs (kits will be present at draw stations)
  - Report confirmatory findings to the newborn screening program at 785-291-3363
- Immunology consultation
  - Lymphocyte proliferation
  - Immunoglobulins
  - Facilitate transfer/admission
- Bone Marrow Transplantation
Immunology

• Dr. Selina Gierer
  – KU Medical Center
  – Kansas City, KS
  – 913-588-5000
  • Page the Immunology faculty/fellow on call

• Dr. Nikita Raje
  – Children’s Mercy
  – Kansas City, MO
  – 816-234-3390
  • Page the Immunology faculty/fellow on call

Additional Information

• Genetics Home Reference

• SCID.net
  http://www.scid.net/

• Immune Deficiency Foundation
  http://primaryimmune.org/