

From the Mind of the Chair



Hello again and welcome to the summer edition of the PMF division newsletter! It is hard to believe that we will be headed to Philadelphia in a few weeks. In this edition, we preview the events, education and research taking place during the 68th AACC Annual Scientific Meeting

& Clinical Lab Expo. Please plan to attend our jointly hosted mixer on Sunday night, following the opening mixer. It will be a great chance to enjoy food and drink while networking with colleagues from the Pediatric and Maternal-Fetal, Industry, Informatics, Clinical Translational Science, and Industry divisions. We will also acknowledge the recipients of our division's awards at this time (read more in this issue). To see the latest science, check out our poster walk and the oral abstracts session featuring work in our areas of interest.

In this issue, we continue our ABC's of Laboratory Medicine with letter 'X', featuring a multi-disciplinary review on "X-linked Severe Combined Immunodeficiency." Excerpt from the Literature summarizes a new report on the utility of decision rules in transcutaneous bilirubin measurements.

Lastly, I want to update you about our division's involvement in AACCs' ongoing advocacy activities. Several members of the PMF division were instrumental in drafting two documents: (1) the new position statement on improving pediatric reference intervals and (2) a policy report focusing on the involvement of the clinical laboratory in children's health. The position statement is currently available on the

AACC website (<https://www.aacc.org/health-and-science-policy/advocacy/position-statements/2016/pediatric-lab-resultsthe-need-for-normal>), whereas the policy report should be available later this summer. AACC will use these pieces in its visits to the Hill and in other communications to inform the public about the issues and importance of our profession.

I hope that you enjoy this edition of the newsletter and that you will continue to join us in our efforts to advance the practice of pediatric and maternal fetal laboratory medicine. See you in Philly!

Shannon Haymond, PhD
Chair, AACC PMF Division

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The ABC's of Pediatric Laboratory Medicine:



X IS FOR "X-LINKED SEVERE COMBINED IMMUNODEFICIENCY"

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Severe combined immunodeficiency (SCID), also known as "Bubble Boy Disease," is a syndrome characterized by profound deficiencies of T, B and sometimes NK cell function. The disorder has gained more attention in recent years with the introduction of a newborn screening test and the need for adequate laboratory work-up to support and confirm the diagnosis. General immunophenotypic and genetic pitfalls are highlighted below.

Introduction:

SCID, like other primary immunodeficiencies, has been linked to the X-chromosome (Table 1), partially explaining the suggested higher incidence of infections among males (1). Though its genetic origins are diverse, it is estimated that approximately 46% of SCID cases in the U.S. are caused by the X-linked form. SCID has an incidence around 1:50,000-100,000 live births and it seems to affect all ethnic groups (2). The disease typically presents in the first year of life and is almost uniformly fatal if not treated early. Patients may initially present with common infections such as otitis media or pneumonia but often develop oral candidiasis, severe viral infections, chronic diarrhea, and/or opportunistic infections (*P. jirovecii*, fungus, mycobacteria) (3). Chronic infection may result in failure to thrive. In countries outside the U.S. where the Bacille Calmette Guerin vaccine is still administered, patients may also present with disseminated mycobacterial infection (4).

SCID is an immunologic emergency. When the diagnosis is confirmed by flow cytometry (see below) and T-cell proliferation assays, patients should be referred to a center with expertise in bone marrow transplant for primary immunodeficiency disorders. While genetic testing should be sent out, transplant should not be delayed for a molecular diagnosis. Precautions should be taken to only transfuse CMV reduced risk blood products that have been irradiated due to the risk of graft-vs-host disease from alloreactive donor lymphocytes (5). Given the risk for opportunistic infections, patients should be started on prophylaxis with trimethoprim/ sulfamethoxazole or pentamidine and should not receive live viral vaccines, including the rotavirus vaccine. Immune globulin replacement (IVIG) is often necessary before and after transplant. Patients and families should be counseled to avoid crowded settings, sick contacts and to limit contact to immediate family members until immune reconstitution. Every effort should be made to identify an HLA-identical donor. If this is not possible, successful bone marrow transplants may be performed with a graft from a T-cell depleted haploidentical donor (6) or a matched unrelated donor (7). Umbilical cord blood transplants have also been performed, albeit, with less encouraging results (8). Gene therapy has also shown promise but is only available in clinical trials (9). Patients who undergo transplant within the first 3.5 months of life have increased survival rates and better immune reconstitution (10). The survival advantages of early transplant have been attributed to a lower incidence of severe infections (10).

Laboratory Analysis:

The newborn screening for SCID has recently been instituted in 45 of 50 states across the U.S. T-cell receptor excision circles (TRECs) produced during T cell development in the thymus can be measured via real-time polymerase chain reaction from punches of dried blood spot cards (11), serving as a surrogate for T cell lymphopenia (12). Newborn screening allows for pre-symptomatic diagnosis and earlier initiation of treatment, which is

particularly helpful for affected infants without a known family history of the disorder. However, an abnormal TREC result in a newborn should be followed by clinical correlation and supportive laboratory analysis since a high rate of false positives is seen, especially in severely ill premature infants (less than 36 weeks gestation). In addition, positive results may be seen in patients with ataxia telangiectasia (13).

As suggested, the characteristic laboratory findings of SCID include profound lymphopenia with low T cells and absent antibody responses on a previously vaccinated infant. Because approximately 70% of the lymphocyte compartment is comprised of T cells, patients with SCID usually have decreased absolute lymphocyte counts. An absolute lymphocyte count <1500 cells/ μ L in an infant should warrant immediate referral and further investigation. This lymphopenia also accounts for the absence of a thymic shadow on chest imaging.

Immunophenotyping:

Immunophenotypic analysis for peripheral blood lymphocyte subset enumeration should be performed in all patients with suspected immune deficiencies (14). Flow cytometric immunophenotyping uses fluorochrome-conjugated monoclonal antibodies against specific antigens to identify and quantitate hematopoietic cells. Lymphoid lineages are defined by expression patterns for certain antigens, typically referred to by cluster designation (CD) number. A basic screening panel will typically include evaluation for total T cells (CD3 positive), helper T (Th) cells (CD3 positive, CD4 positive), cytotoxic T (Tc) cells (CD3 positive, CD8 positive), natural killer (NK) cells (CD16 and/or CD56 positive, CD3 negative), and B cells (CD19 or CD20 positive). The lymphocyte subset numbers are compared to age appropriate reference intervals (15) and should account for the developmental stage of the patient's immune system. This analysis is abnormal in almost all cases of SCID.

In SCID the immunophenotype is often an indicator of the genetic defect. The most common phenotype observed in SCID is a marked reduction in total T cells (including Th and Tc subsets) with a moderate to marked decrease in NK cells and normal numbers of B cells, although the latter are nonfunctional (16). This phenotype is observed in X-linked SCID and also autosomal recessive SCID with mutations in Janus 3 (*Jak3*) kinase. Other forms of SCID possess characteristic phenotypes. Defects in V(D)J recombination, required for B cell and T cell maturation, result in a SCID phenotype with markedly decreased B cells and T cells but preservation of NK cell numbers. These V(D)J recombination defects (see Genetics section below) include, but are not limited to mutations in *RAG1*, *RAG2*, *DCLRE1C* (Artemis) (17), and *PRKCD* (ref Woodbine). Loss of T cells (including Th and Tc subsets) with preservation of B cells and NK cells is associated with mutations of interleukin 7 receptor alpha (*IL7RA*), coronin 1a (*CORO1a*), *CD45*, and *CD3* (16-18). Reduction in all lymphoid subsets is typical of adenosine deaminase (*ADA*) and adenylylase kinase-2 (*AK2*) mutations (16-18). Isolated absence of circulating Tc is suggestive of zeta-associated-protein 70 (*ZAP-70*) mutation (19). Finally, T-cell proliferation in response to antigens and mitogens is nearly absent in almost all forms of SCID (3). Transplacental transfer of maternal IgG in utero may result in normal levels of Ig in SCID during the first 6 months of life. However, IgA and IgM levels are usually depressed.

Genetics:

Diagnostic confirmation is obtained by genetic analysis. Of interest, SCID mutations have a higher incidence in certain ethnic populations. Adenosine Deaminase-SCID (ADA-SCID) is seen in the Somali population with an incidence of ~1 in 5,000; *DCLRE1C* (Artemis) mutations occur in Navajo Americans at a rate of ~1 in 2,000; and *RAG1*, *RAG2*, *ADA*, *IL7RA*, *CD3*,

and *ZAP70* mutations have a similar incidence in the Amish and Mennonite populations.

X-linked SCID is caused by mutations in the *IL2RG* gene, encoding the common gamma chain (γ_c), a component of a cytokine receptor. The mutation is seen in approximately 19% of SCID cases screened in this country (13). The gene is composed of 1,124 nucleotides spread out over 8 exons, and the protein product is 389 amino acids long. Over 200 pathogenic *IL2RG* mutations have been reported, with the most common being loss of function missense mutations (20). Several hotspot variants have been reported, including at codons 224, 226, 285 and 289 (21). About 1% of *IL2RG* mutations are thought to be partial or whole gene deletions (13). Mutations that do not cause loss of function are more likely to lead to atypical SCID, which is $T^+B^+NK^-$, and can present as an attenuated phenotype (22).

X-linked SCID follows an X-linked recessive inheritance pattern; females with pathogenic mutations remain unaffected carriers and only males become symptomatic. The mothers of affected males are not obligate carriers, as it is possible for an *IL2RG* mutation to occur *de novo*. A carrier female has a 50% chance of having an affected son, and a 50% chance of having a carrier daughter. An affected male has no chance of having an affected son, and a 100% chance of having a carrier daughter.

Summary:

X-SCID is an immunologic emergency that requires a multidisciplinary diagnostic and therapeutic approach. Newborn screening is available at most US states and the disorder is more prevalent in certain ethnic groups.

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Table 1: X-linked primary immunodeficiencies (1)

Disease	Gene	Females	Males
X-Linked Agammaglobulinemia (XLA)	BTK	Usually not affected	Absence of mature B-cells and immunoglobulins; severe immunodeficiency and susceptibility to microbial infections.
Wiskott-Aldrich Syndrome (WAS)	WAS	Usually not affected	Absence of T-cells and platelets; severe immunodeficiency and susceptibility to microbial infections; eczema.
X-linked Neutropenia and Myelodysplasia	WAS	Usually not affected	Severe congenital neutropenia and monocytopenia; recurrent bacterial infections.
X-linked Chronic Granulomatous Disease (X-CGD)	CYBB	Reduction in superoxide; higher incidence of SLE;	Deficient superoxide production in phagocytes; severe bacterial and fungal infections; colitis.
Properdin Deficiency	PFC	Low to normal levels of properdin	Absent hemolytic activity by the complement alternate pathway; susceptibility to meningococcal (<i>Neisseria spp.</i>) infections.
Immunodysregulation, polyendocrinopathy and enteropathy; X-linked syndrome (IPEX)	FOXP3	Usually not affected	Enteropathy; insulin-dependent diabetes mellitus; dermatitis, thyroiditis, hemolytic anemia; thrombocytopenia; eczema; recurrent infections.
X-linked Severe Combined Immunodeficiency (X-SCID)	IL2RG	Usually not affected	Absence or diminished numbers of T-cells and natural killer cells; non-functional B-cells; decreased levels of immunoglobulins; severe susceptibility to infections.
X-linked Lymphoproliferative Disease Type 1 (XLP1)	SH2D1A	Usually not affected	Severe EBV infection; aplastic anemia; reduced number of B-cells; infectious mononucleosis syndrome.
X-linked Lymphoproliferative Disease Type 2 (XLP2)	XIAP	Usually not affected	Splenomegaly; bone marrow failure; increased apoptosis of peripheral blood lymphocytes.
CD40L Deficiency (X-linked Hyper-IgM Syndrome)	CD40L	Usually not affected	Low levels of IgG, IgE, and IgA with high levels of IgM; neutropenia; thrombocytopenia; hemolytic anemia; recurrent infections.
Anhidrotic Ectodermal Dysplasia with Immunodeficiency (EDA-ID)	IKBKG	Usually not affected	Dysgammaglobulinemia; susceptibility to infections; defective NF-κB signaling.
Incontinentia Pigmenti	IKBKG	Skin, hair and teeth abnormalities	Usually death in utero; male survivors have severe immunodeficiencies and susceptibility to infections
Glucose-6-phosphate Dehydrogenase (G6PD) Deficiency	G6PD	Usually not affected	Deficient superoxide production in phagocytes; severe hemolytic anemia.

Excerpt from the Literature



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Utility of Decision Rules for Transcutaneous Bilirubin Measurements

Taylor JA, Burgos AE, Flaherman V, Chung EK, Simpson EA, Goyal NK, Von Kohorn, Dhepyasuwan N; BORN Investigators. *Pediatrics*. 2016 May; 137(5).

During hospitalization of newborns, it is important to identify those with significant hyperbilirubinemia. Therefore systematic screening of newborns for hyperbilirubinemia is commonly performed for this purpose. Screening for hyperbilirubinemia by transcutaneous bilirubin (TcB) measurement is advantageous as it is a non-invasive point-of-care method with acceptable performance and results are immediately available. As a screening tool, TcB is used in conjunction with decision rules which have defined bilirubin threshold values above which a blood draw for a confirmatory total serum bilirubin (TSB) test is indicated. Different institutions may employ different decision rules when using TcB to screen for hyperbilirubinemia which may include either of the three decision rules suggested by the American Academy of Pediatrics (AAP).

The goal of this publication was to evaluate the clinical utility of three TcB screening decision rules: $\geq 75^{\text{th}}$ percentile on the Bhutani nomogram, 70% of the phototherapy level, and within 3 mg/dL of the phototherapy threshold. The authors aimed to identify one (or more) robust decision rule with a false-negative rate

close to 0% (corresponding to a sensitivity near 100%) in detecting newborns with an elevated TSB concentration, while removing the need for a blood draw in most infants. They included two sample groups of patients: Sample 1 included 911 paired TcB-TSB measurements from newborns at nursery sites where screening with TcB measurements was routine and TSB measurements obtained within 2 hours of the TcB when indicated. Because the study design in Sample 1 could lead to overestimation of the sensitivity of TcB, a second sample set (Sample 2) with a different study design was assessed in an attempt to account for this possibility. Sample 2 included 913 paired TcB-TSB measurements from newborns at nursery sites where screening with TSB measurements was routine and a paired TcB was prospectively obtained at the time of the blood draw.

The authors found that during birth hospitalization, the use of specific decision rules for TcB measurement can be effective when screening newborns for hyperbilirubinemia. The study did not identify any one decision rule that outperformed the others. However, it provides more understanding of the strengths and weaknesses of each of the three decision rules evaluated. With respect to false-negative rates, there were no significant differences amongst the three decision rules in both sample groups. Even though the need for blood draws for TSB measurements after ~ 80 to 90% of TcB measurements was eliminated in Sample 1, the study pointed out that some of the decision rules would lead to a blood draw significantly more often than other rules evaluated. Since none of the decision rules evaluated identified all newborns with actionable TSB concentrations, clinical judgement as to whether a TSB measurement is needed following TcB measurement is important. Laboratorians should be aware of the decision rules utilized at their institutions, collaborate with clinical teams to identify appropriate turnaround times, and work to provide TSB measurements in a timely manner in situations where confirmatory testing is needed.

2016 AACC Annual Scientific Meeting and Clinical Lab Expo: PMF Sessions of Interest and Meeting Highlights

JULY 31-AUGUST 4, 2016 IN PHILADELPHIA, PENNSYLVANIA

Sunday, July 31st

Opening Plenary:

John McDevitt, PhD

The Programmable Bio-Nano-Chip: A Platform to Digitize Biology.

Monday, August 1st

Brown Bag Sessions:

Diagnosis and Monitoring of Congenital Adrenal Hyperplasia, Polycystic Ovarian Syndrome and Pubertal Abnormalities Using Mass Spectrometry. 42111 & 52211.

Prenatal Screening for Down Syndrome: From Maternal Serum Screening to Cell-Free Fetal DNA Testing. 42117 & 52217.

Nonfasting Lipid Profiles. 42129 & 52229.

Tuesday, August 2nd

Brown Bag Sessions:

Therapeutic Drug Management in Pregnant Patients. 43104 & 53204.

Case Study Based Approach of Prenatal Maternal Double Marker Screening: Save the Precious Little Foot Prints in Mother's Womb. 43123 & 53223.

Oral Abstract Session:

Breakthroughs in Maternal, Fetal and Pediatric Medicine. 33101.

Wednesday, August 3rd

Brown Bag Session:

Laboratory Assessment of Cystic Fibrosis. 44108 & 54208.

Afternoon Symposia:

Precision Medicine Delivered by Advances in Circulating Cell-Free DNA Diagnostics. 34212.

Please Join Us!

Event: Pediatric and Maternal-Fetal, Industry, Informatics, Clinical Translational Science, and Industry Divisions Joint Mixer

Date: Sunday, July 31, 2016

Time: 7:30pm-9:00pm

Location: Philadelphia Marriott Downtown-Independence Ballroom - Salon II

Relax and enjoy food and drinks in the company of your PMF colleagues!

Event: Pediatric and Maternal-Fetal Poster Walk. Dr. Brenda Suh-Lailam will guide through highlights in PMF research

Date: Wednesday, August 3, 2016

Time: 12:30pm-1:30pm

Location: Pennsylvania Convention Center

PMF Division Awardees

Please help us congratulate the winners of this year's PMF Division Awards. The awards will be presented during the Pediatric and Maternal-Fetal, Industry, Informatics, Clinical Translational Science, and Industry Divisions Joint Mixer on **Sunday July 31, 2016 from 7:30pm-9:00pm at the Philadelphia Marriott Downtown Hotel.**

Best Abstract by a Student or Young Investigator:

- **David Lin , PhD.** ARUP Laboratories. Salt Lake City, UT. USA
- Title: Tanner Stage-Stratified Pediatric Reference Intervals for Dihydrotestosterone (Abstract B-220)

Best Abstract:

- **Ioannis Papassotiriou, PhD.** Agnia Sophia Children's Hospital. Athens, Greece
- Title: Evaluation of GDF-15 and YKL-40 as Early Markers of Subclinical Diabetic Nephropathy and Cardiovascular Morbidity in Young Patients with Type 1 Diabetes Mellitus (Abstract B-232)

Outstanding Contributions to Pediatric Materna-Fetal Laboratory Medicine:



Ann Gronowski, PhD, DABCC, FACB, Professor, Dept. of Pathology & Immunology
Washington University School of Medicine, Saint Louis, Missouri, USA

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