PEDIATRIC AND MATERNAL-FETAL DIVISION NEWS
Volume 41 | Issue 2 |July 2022

AACC Better health through laboratory medicine.

FROM THE MIND OF THE CHAIR

I hope everyone is enjoying summer and looking forward to the AACC Annual meeting beginning on July 24th. The PMF Division will have a table at the Division Networking Event after the opening plenary session, so please come by and find out about opportunities to become involved in the division. The PMF Division is also co-hosting a Mixer with the Health Equity and Access Division on Sunday night. Join us for food and drinks and networking with your colleagues. Congratulations to our abstract award winners and the Outstanding Contribution to Pediatric and Maternal-Fetal Clinical Chemistry award winner who are announced in this newsletter. They will also be recognized at the mixer.

In this issue we are up to J in The ABC's of Pediatric Laboratory Medicine, and J is for Juvenile Idiopathic Arthritis. Excerpts from the Literature discusses inconsistencies among pediatric reference intervals. Also included is an extensive list of sessions that might be of interest to our members. Thank you to the newsletter team for making it easy to plan out our annual meeting schedule.

This is my last newsletter as the Chair of the division, and I want to thank you all for the honor and privilege of holding this position. The Division will be in great hands with the incoming Chair, Dr. Stanley Lo, and I couldn't hand the baton to a better person. The election for other positions on the PMF Executive board is open until July 19th, so please vote if you have not already done so.

Sincerely,
Angela Ferguson, PhD
Chair, AACC PMF Division

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THE ABC'S OF PEDIATRIC LABORATORY MEDICINE:

J is for Juvenile Idiopathic Arthritis: One Term, Many Entities

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Background

Juvenile idiopathic arthritis (JIA), formerly known as juvenile rheumatoid arthritis, is one of the most common rheumatologic conditions affecting children. Its prevalence in children younger than 16 years has been estimated at between 57-113 per 100,000, however accurate estimates are complicated by the heterogeneous presentation of the disease [1]. The most common age of onset is between 1-3 years with a second peak occurring in boys around 9 years of age [2].

JIA is not a single disorder, but rather it is a term used to describe a wide array of arthritides presenting in children younger than 16 years. Categories of JIA include systemic JIA, rheumatoid factor (RF) positive, enthesitis-related, and oligoarthritis among others. Classification is based on many factors including pattern of joint involvement, presence of autoantibodies, and human leukocyte antigen (HLA) type. While the exact pathogenesis is unknown, emerging evidence suggests that each subtype may be the result of unique immunogenic causes [3].

Diagnosis and Classification

The general definition of JIA is joint inflammation developing in a child younger than 16 years of age with symptoms lasting more than 6 weeks. Currently, the American College of Rheumatology recognizes four distinct JIA subtypes. Systemic JIA (sJIA) typically presents with fevers and rash with progression to joint swelling and, in some cases, internal organ inflammation. Oligoarticular JIA presents with involvement of < 5 joints initially. A subset of patients may develop iritis or uveitis, however other systemic symptoms are less common. Polyarticular JIA affects $\geq$ 5 joints and includes RF positive and psoriatic arthritis. Enthesitis related arthritis (ERA) is a form of spondyloarthritis associated with inflammation of the spinal ligaments [4].

Diagnosis is based on a combination of patient history, physical exam findings, laboratory results, and imaging tests. Exclusion of other entities is important as symptoms often mimic those seen in other conditions including infections, malignancy, connective tissue diseases, vasculitis, and other autoinflammatory disorders. Assessment of biomarker expression is an area of ongoing research with the hope of leading to improved diagnosis and treatment [5].

Laboratory's Role

In addition to ruling out many of the entities described above, the laboratory plays a critical role in the diagnosis and management of JIA patients. Current treatment algorithms are based on validated measures of disease including assessment tools such as ACR provisional criteria for inactive disease and Juvenile Arthritis Disease Activity Score (JADAS) [6]. Erythrocyte sedimentation rate (ESR) is incorporated into both of these tools, while C-reactive protein is only incorporated into ACR provisional criteria [7,8].

The ESR is a commonly performed laboratory test used as a representative assessment of inflammatory activity. It is based on the Westergren method which takes a sample of anticoagulated blood and measures how far the red blood cells (RBCs) fall to the bottom over a

period of one hour. Automated testing systems have been developed which greatly reduce the turnaround time, however the Westergren method still remains the gold standard off of which all current methods are based [9]. The functionality of the test is due to the fact that erythrocytes carry a negative charge on their surface which causes them to repel each other under normal physiologic conditions. Many inflammatory markers are positively charged proteins, and their presence blunts these repulsive forces causing the erythrocytes to clump and fall out of the plasma [10]. Unfortunately, ESR is not a very specific test and results can be affected by many factors other than inflammation. ESR may be falsely elevated in cases of macrocytosis and anemia, and falsely decreased in polycythemia. Abnormally shaped RBCs such as spherocytes and sickle cells can inhibit aggregation and lead to falsely low results. Presence of anti-RBC antibodies can also interfere with aggregation. Albumin and fibrinogen are two positively charged plasma proteins that can effect ESR, and hypoalbuminemia and hypofibrinogenemia may lead to falsely low results [10].

Sample preparation can also affect ESR. For best results, blood should be kept at room temperature, run within 2 hours of collection, and testing equipment should be checked to make sure that it is not placed in an area where it may be exposed to vibration. Because there are so many factors that can affect a single ESR value, many physicians find greater clinical utility by monitoring ESR trends over time [10].

In addition to an elevated ESR, other laboratory findings suggestive of an inflammatory response are also frequently detected. These can include increased C-reactive protein, leukocytosis, thrombocytosis, and increased ferritin levels. Positive rheumatoid factor (RF) may be suggestive of RF-positive polyarticular JIA, however if seen in the presence of a positive antinuclear antibody (ANA), other autoimmune conditions should be considered such as Sjogren's syndrome or mixed connective tissue disease [11]. Positive ANA by itself has been seen in the oligoarticular subtype, and is frequently regarded as a risk factor for development of uveitis [12].

Though not commonly tested for in the clinical laboratory, many additional biomarkers have been studied in relation to JIA. Increased serum MMP-3 has been found to be associated with enthesitis-related arthritis. Inflammatory S100 proteins may be useful in predicting response to various therapies. More specific laboratory markers, such as IL-18, are being investigated as potential replacements for ESR to better measure treatment response. Soluble IL-2 receptor, CD163, and follistatin-like protein 1 are additional biomarkers that have been suggested to play a role in disease progression and response [5].

Management

Current management of JIA is largely dependent on whether the condition is manifesting as oligoarticular or systemic JIA. In cases of oligoarticular JIA, intra-articular glucocorticoids are strongly recommended as a first-line treatment with the possible addition of NSAIDs [6]. Response to therapy should be monitored using a tool such as ACR provisional criteria or JADAS, both of which include normalization of ESR as a criterion suggesting a positive treatment response [7,8]. If resolution is not achieved, then advancing treatment to include biologic and non-biologic disease-modifying antirheumatic drugs (DMARDs) such as methotrexate or hydroxychloroquine is recommended [6].

Management of systemic JIA depends on the presence or absence of signs indicative of macrophage activation syndrome (MAS), a subtype of hemophagocytic lymphohistiocytosis (HLH) that can be seen in pediatric rheumatologic conditions. Multiple criteria for identification of MAS in the setting of sJIA have been proposed. The criteria put forth by Ravelli et al are a commonly referenced example (Table 1) [13].

In cases of sJIA with MAS, the recommended first-line treatment is an IL-1 or IL-6 inhibitor with or without systemic glucocorticoid treatment. If arthritis symptoms do not resolve, then adding or switching to another DMARD is recommended.

Once disease resolution is achieved, tapering and eventual cessation of glucocorticoid and DMARD therapy is recommended. Management of sJIA without MAS is similar with the only difference being that glucocorticoids are not recommended. Instead, a brief trial of NSAIDs along with an IL-1 or IL-6 inhibitor is the recommended first-line of therapy [6].

Table 1: Criteria for identification of macrophage activation syndrome (MAS) in sJIA[a]

Febrile patient with suspected systemic Juvenile Idiopathic Arthritis (sJIA)
Ferritin level > 684 ng/ml
At least two of the following:
a. Platelet count $\leq$ 181 x 10^9/liter
b. Aspartate aminotransferase (AST) > 48 units/liter
c. Triglycerides > 156 mg/dl
d. Fibrinogen $\leq$ 360 mg/dl

[a] Table from Ravelli et al [13]

Prognosis

Uveitis is one of the most common extra-articular manifestations of JIA, however it is usually only seen in oligoarticular and enthesitis related subtypes. Various risk factors for development of uveitis have been identified, including an ESR > 22 mm/h and age < 3 years at time of diagnosis. Time of uveitis onset is typically within 4 years of JIA diagnosis. For this reason, regular ophthalmology screenings are recommended for JIA patients [14].

Overall prognosis was poor prior to the development of modern therapy, with nearly two-thirds of untreated patients continuing to experience persistent disease 10 years after diagnosis. The introduction of DMARDs has led to significant clinical improvements in treated patients [15]. It has been suggested that while ANA status may not be predictive of disease outcome, the presence of other high-titer nuclear antigen autoantibodies may suggest a poor response to current treatment regimens. Additionally, the presence of RF, HLA-B27, and uveitis have been suggested as independent predictors of worse prognosis [16].

Conclusion

Juvenile idiopathic arthritis encompasses a collection of subtypes that demonstrate unique physical and laboratory findings. The laboratory plays an essential role in the diagnosis, classification, and monitoring of disease with involvement by numerous disciplines including clinical chemistry, hematology, and immunology. ESR is a common laboratory test that has been incorporated into most JIA diagnostic and management criteria. As our understanding of disease pathophysiology and treatment expands, the clinical laboratory will likely play an even greater role in the future.

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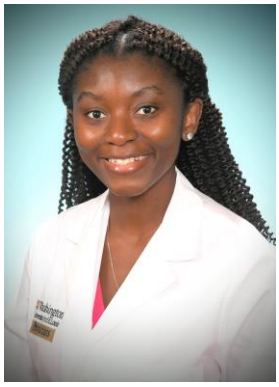
Excerpts from the Literature



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Inconsistencies among pediatric reference intervals

Reference intervals provide a framework for interpreting laboratory-generated biomarker concentrations and are critical for clinical decision making. Pediatric reference intervals (PRIs), in particular, can create confusion and have a negative impact on clinical judgment if they do not adequately reflect the biochemistry of normal human development. A recent systematic review entitled “Current state of pediatric reference intervals and the importance of correctly describing the biochemistry of child development: A Review”, by Lyle and colleagues, published online in JAMA Pediatrics, assessed the consistency of PRIs from different sources [1]. The authors noted a wide variation in the patterns, magnitude, and timing of biomarker changes among the various PRIs.

The concentrations of numerous biomarkers are dynamic from infancy through puberty. Thus, while absolute values of PRIs are expected to vary based on the choice of analytical system and QC materials, differences and patterns related to normal physiology should endure. The authors compared PRIs for 8 commonly measured analytes: free thyroxine (FT4), thyrotropin, cystatin C, insulin like growth factor 1 (IGF-1), testosterone, estradiol (E2), hemoglobin, and ferritin. PRIs were obtained from 9 clinical practices/hospitals, literature searches on PubMed, large commercial laboratories, the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER), and Children’s Health Improvement through Laboratory Diagnostics (CHILDx) study cohorts.

The PRIs for each biomarker studied were all inconsistent. The most significant PRI inconsistencies were observed for biomarkers with rapid changes in concentration, e.g., FT4 and thyrotropin during the first year of life, or sex hormones with the onset of puberty. This review identified a number of causes of the inconsistent PRIs. Three major contributors that affected all the biomarkers assessed are discussed below.

A major contributor to the discrepancies was the adoption of an inconsistent number and range of age groups for establishing PRIs. The assessed biomarkers had a variable number of age bins, ranging from 1 to 12 bins for hemoglobin and ferritin, 2 to 8 bins for FT4, 1 to 7 bins for E2, and 1 to 12 bins for testosterone. Further, the age range for each bin varied significantly for each measurand. For instance, three age bins were employed by 5 of the 21 PRIs for FT4. The age ranges for those bins, however, were varied. For example, in the youngest bin, the 5 PRIs assessed used 0 to 2 weeks, 0 to 3 days, 0 to 20 days, 1 to 12 months, and 0 to 5 years as binning

strategies. Interestingly, 4 of the 21 FT4 PRIs were generated from the same CALIPER cohort using different assays, and a different number and range of age groups, leading to different PRIs. Because of this heterogeneity, some of the PRIs failed to reflect the expected increase in FT4 in the first few days of life. Additionally, not all the biomarkers were sub-stratified by sex or Tanner stage.

Another significant contributor was the use of different inclusion/exclusion criteria for PRI determination. Almost all of the studies omitted the inclusion and exclusion criteria that were used to guarantee a healthy representative population. The CHILDx study, for instance, was the only study that described the exclusion of participants with thyroid peroxidase autoantibodies from FT4 PRI calculations, thus more likely reflecting a population with a normal thyroid axis. Furthermore, despite the fact that E2 and total testosterone concentrations are known to vary with the menstrual and diurnal cycles, respectively, none of the PRIs for total testosterone and E2 included information on the time of day or point in the menstrual cycle at which samples were obtained.

Further, the number of participants per age grouping was not disclosed for several PRIs. The Clinical Laboratory Standards Institute Guidelines EP-28 recommends having a minimum of 120 healthy participants per group, yet several age groups in the studies who reported PRIs participant number failed to reach this requirement. PRIs for age groups younger than 12 months of age were more likely to have been generated from fewer than 120 participants.

The findings of this review suggest that some PRIs do not represent typical physiological changes. The recruitment of healthy study participants is laborious, the lack of assay standardization, and other factors make the establishment of PRIs logistically and analytically difficult. Key stakeholders working to eliminate PRIs inconsistencies include the CDC and AACC, whose goals are to generate and disseminate continuous PRIs to improve pediatric clinical care.

References

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2022 AACC ANNUAL SCIENTIFIC MEETING & CLINICAL LAB EXPO: PMF Sessions of Interest and Meeting Highlights

July 24-28, 2022

Sunday July 24th

Opening Plenary:

Lucila Ohno-Machado, MD, PhD, MBA
11001 Biomedical Informatics Strategies to Enhance Individualized Predictive Models

PMF Division Mixer co-hosted with the Health Equity & Access Division

8:00-9:30 PM Hyatt Regency McCormick Place, Prairie A. There will be food and everyone will receive one drink ticket. Awards will be presented during this time.

Monday July 25th

Roundtables:

42106 and 52206 Children Are Not Little Adults: Special Considerations in Pediatric Laboratory Testing

42110 and 52210 Establishment of Pediatric Reference Intervals

42117 and 52217 Introduction to Biochemical Genetics from the Clinical Laboratory Perspective: A Case-Based Discussion

42120 and 52220 Modalities to Work Up Hemoglobinopathies

Plenary:

George Church, PhD
12001 Multiplexed and Exponentially Improving Technologies

Scientific Sessions:

32110 Preanalytical Challenges of Blood, Sweat, and Urine Collection in Pediatric Populations

32230 Valid Vital LDTs: Current State of Regulation Legislation of Laboratory-Developed Tests

Tuesday July 26th

Roundtables:

43112 and 53212 Estimated GFR in Children: New Equations and Clinical Applications of Cystatin C

43113 and 53213 Exploring Current and Emerging Novel Laboratory Biomarkers of Preeclampsia

43129 and 53229 You're Gonna Need a Smaller Tube: Necessary Considerations in Pediatric Laboratory Medicine

Plenary:

Alysson Muotri, PhD
13001 Applications of Human Brain Organoid Technology

Scientific Sessions:

33101 AACC Guidance on the Use of Point-of-Care Testing in Fertility and Reproduction

33230 Unusual Toxicology: Interpreting Complex Cases Involving Urine, Umbilical Cord, Meconium, and Hair Samples

33232 Laboratory Testing for the Assessment of Preterm Delivery: A Summary of the AACC Academy Guidance Document

Wednesday July 27th

Roundtables:

44102 and 54202 Analytical and Clinical Evaluation of sFlt-1 and PlGF Immunoassays for Predicting Preeclampsia

44106 and 54206 Cases in Pediatric Clinical Chemistry

44120 and 54220 Molecular Diagnostics Profoundly Alters Clinical and Life-Changing Outcomes in Diagnosing Inherited Metabolic Disorders

Plenary:

Thomas Lee, MSc, MD
14001 Building Trust in a Time of Turmoil

Scientific Sessions:

34111 Testing Strategies for Detecting Pediatric Drug Exposure: A Case Based Discussion

34224 Do Pediatric Reference Intervals Reflect the Development of Healthy Children?

34226 Neonatal Drug Testing: Pick Your Candidate!

Thursday July 28th

Plenary:

Livia Eberlin, PhD
15001 Guiding Clinical Decisions with Molecular Information provided by Direct Mass Spectrometry Technologies

PMF Division Awardees

Please help us congratulate the winners of this year's PMF Division Awards!

Best Abstract by a Student or Young Investigator:

Mary Kathryn Bohn
PhD Candidate
University of Toronto
Title: Serological Antibody Response to SARS-CoV-2 Vaccination in a Large Cohort of Canadian Children, Adolescents, and Adults

Best Abstract:

Gustavo Barra, PhD
Sabin Medicina Diagnostica
Title: Incorporating spinal muscular atrophy screening by next-generation sequencing into a comprehensive multigene panel for newborn sequencing: a pilot evaluation

Outstanding Contributions to Pediatric Maternal-Fetal Laboratory Medicine:

Nathalie Lepage, PhD, FCCMG, FCACB
Laboratory Head, Biochemistry – Newborn Screening Ontario
Children's Hospital of Eastern Ontario

Full Professor
Department of Pathology and Laboratory Medicine
University of Ottawa

PMF Division Executive Board:

Thank you to our division officers who will be completing their terms this month. They are:

Past Chair
Alison Woodworth, PhD

Secretary
Mark Kellogg, PhD

Treasurer
Joe Wiencek, PhD

Members At Large
Amy Karger, PhD
Jane Dickerson, PhD

Fellow Representative
Erin Schuler, PhD

Elections have not yet been completed for new officers. Our remaining officers are:

Chair
Stanley Lo, PhD

Chair Elect: TBD

Past Chair
Angela Ferguson, PhD

Members At Large
Van Leung-Pineda, PhD
Laura Smy, PhD

Secretary: TBD
Treasurer: TBD

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