PEDIATRIC AND MATERNAL-FETAL DIVISION NEWS
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AACC Better health through laboratory medicine.

FROM THE MIND OF THE CHAIR

Dear Friends,

Happy New Year!

In December, NIH Director Dr. Francis Collins announced the closure of the National Children's Study (http://nih.gov/about/director/12122014_statement_ACD.htm).The decision was based on conclusions from the working group and certainly felt like a piece of coal in our stocking (http://acd.od.nih.gov/reports/NCS_WG_FINAL_REPORT.pdf). On the bright side, the news was attenuated by Dr. Collins'statement that the agency will make the collected data and specimens available to researchers. More importantly, it appears that the study failed to meet expectations due to methodological issues. Does this open up an opportunity for laboratory professionals to continue our mission on this noble project? I can assure you that our group is interested in all future projects that support these goals.

The start of the New Year means new horizons for our Division. We continue our long tradition of education, advocacy and service dedicated to expectant mothers and children. Our current newsletter feature highlights the fact that "some things are worth their weight in gold!" Urine samples are a very valuable sample in children and if you have any doubts, Dr. Marvin Natowicz's and Dr. Brad Karon's comprehensive review on this issue will certainly make you reconsider all the possibilities. We also have a wrap-up of the IFCC's triennial Pediatric Laboratory Medicine 2014 meeting in Istanbul, an interview with newly elected AACC President and PMF veteran, Patti Jones, and more.

Finally, let me thank you all for participating in our recent board elections and please congratulate the elected members at large Dr. Jon Nakamoto and Dr. Angela Ferguson, our Treasurer Dr. Sihe Wang and our new fellow Dr. John Mills.

Please let us know your thoughts. We would like to hear your needs, suggestions, new ideas or anything related to our scope of activities. We are here to help you make a difference in the life of all patients.

Best,

David Carpentieri, MD

Chair, AACC PMF Division

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PEDIATRIC AND MATERNAL-FETAL DIVISION ELECTION RESULTS

Thank you to all who participated in the recent election. The results are as follows:

Member at Large for 2015-2017

Dr. Jon Nakamoto
Dr. Angela Ferguson

Treasurer for 2015-2016
Dr. Sihe Wang

Change to the Bylaws
Approved

The PMF Division welcomes our second Fellow, Dr.John R. Mills, to the Division's Executive Management team

Dr.Mills is a Clinical Chemistry Fellow in the Department of Laboratory Medicine and Pathology at Mayo Clinic. He received a B.Sc. in biochemistry and a Ph.D. in biochemistry and chemical biology from McGill University, Montreal, Canada. A member of AACC (2012-present), SYCL (2013-present) and the Canadian Society of Clinical Chemists (CSCC, 2013-present), his research focuses on the application of mass spectrometry to improve diagnosis and monitoring of multiple myeloma. Dr. Mills is interested in the clinical application of next generation sequencing and begins a two year training program in clinical molecular genetics at Mayo Clinic this year. We look forward to having Dr. Mills join our group.

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THE ABC'S OF PEDIATRIC LABORATORY MEDICINE-U IS FOR URINE

Clinical Mass Spectrometry of Urine for the Screening of Selected Inborn Errors of Metabolism

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Pathology & Laboratory Medicine, Genomic Medicine, Pediatrics and Neurology Institutes
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There are hundreds of known monogenic metabolic disorders in humans. The Human Genome Project and its detailed description of the human genome has recently facilitated the prediction of many more inborn errors of metabolism and, due to the increasing utilization of whole exome and whole genome sequencing in both clinical and research settings, new single gene metabolic disorders are now discovered weekly.

The screening and diagnosis of inborn errors of metabolism usually requires the judicious selection of diagnostic tests and of the tissue(s) to be assayed. Urine is the tissue of choice for screening for some families of metabolic disorders and is a useful ancillary tissue for screening for many others. In this brief note I review two key uses of mass spectrometric analyses of urine in screening for several families of genetic metabolic disorders, genetic disorders of organic acid metabolism and inborn errors of bile acid synthesis.

Organic acids are physiological intermediates in many metabolic pathways, including in the metabolism of amino acids, fatty acids, carbohydrates, purines, pyrimidines and some neurotransmitters. Genetic disorders causing a significant inefficiency or absence of an enzyme needed for the metabolism of organic acids typically results in a pathologic excess of one or more organic acids in the body. These excess metabolites are excreted in the urine, the tissue of choice for screening for most disorders of organic acid metabolism. A pathological excess of one or more organic acids can also occur when a key enzyme of organic acid metabolism is rendered inefficient on a secondary basis due to an inhibition of its enzyme activity such as a result of a genetic abnormality of an unrelated enzyme or when there is reduced enzymatic function because of an inadequate level of a needed vitamin co-factor or other nutrient. Abnormal urinary organic acid patterns can also be observed due to intake of some non-standard diets

and certain infant formulas or consequent to alterations of the gut microbiome as can be seen in short gut syndrome or other intestinal pathologies.

The most common families of disorders screened for by urinary organic acid analysis are the organic acidurias. The organic acidurias that are commonly screened for are mainly due to defective function of enzymes involved in the downstream metabolism of many amino acids; others are due to selected vitamin dysmetabolisms. Some examples of these disorders include the methylmalonic acidurias, propionic aciduria, isovaleric aciduria, glutaric aciduria types I and II, multiple carboxylase deficiency and biotinidase deficiency, although there are many others. Urinary organic acid analysis is also an important tool, though usually not the primary assay, to screen for disorders of mitochondrial fatty acid oxidation and disorders of mitochondrial oxidative phosphorylation.

The clinical presentations and natural histories of the organic acidurias are highly variable. One important phenotype is the neonatal onset form that presents with irritability, lethargy and vomiting within a few days after birth and which can rapidly progress to obtundation, seizures and coma if not recognized and promptly treated. Later onset forms, including forms with onset in later childhood, adolescence and, sometimes, in the adult years are well-recognized, too. Common to most clinical phenotypes is a dietary intolerance to excess amounts of the amino acid(s) that is a precursor to the accumulating organic acid. Common to all phenotypes is a risk of general metabolic and neurological deterioration in the context of increased energy demands and catabolic stresses. When ill, patients typically have an anion gap acidosis and ketonuria. Variable hypoglycemia, lactic acidemia and mild-moderate hyperammonemia are other common biochemical findings, especially during times of metabolic decompensation. An important rule in clinical metabolism is that an inborn error of metabolism should be strongly considered in any neonate with unexplained marked ketonuria. There are excellent clinical reviews of the organic acidurias (1, 2).

From a diagnostic perspective, analyses of blood and urine specimens when the patient is acutely ill and before treatment has been initiated provides the best opportunity for diagnosis; the concentration of the diagnostic metabolites is highest at that time as the levels of these compounds lessen once proper treatment is begun. It is often useful to analyze leftover clinical specimens from the time of presentation in the emergency department if no diagnosis has been achieved and early samples were not analyzed. Despite use of good analytic methods, a diagnosis can sometimes be missed if a non-acute sample is analyzed or if a patient has a vitamin-responsive form or a variant clinical form with substantial residual enzyme activity (i.e., a ‘leaky’ mutant).

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The vast number of urinary organic acids, with their marked variation in physical properties, makes clinical urinary organic acid analysis challenging. It is generally performed by capillary gas-liquid chromatography/mass spectrometry of trimethylsilyl derivatives with identification and quantification by computer-based libraries that utilize both retention time and mass spectra indices. Other less commonly used analytic methods include liquid chromatography-chemical ionization mass spectrometry or automated capillary electrophoresis. The extraction of organic acids from urine is a critical step because of the varied recoveries of the organic acids; multiple methods are in use with most labs using a solvent extraction protocol. Some organic acids require different derivatization procedures or stable isotope dilution assays. An overview of preanalytical considerations and the analytical aspects of urinary organic acid analysis can be found in several excellent reviews (3, 4). Regardless of the analytical protocol used, the ideal performance of this assay involves good communication of clinical information from the care providers and of the clinical laboratory findings from the clinical laboratory professional.

The second test to be reviewed in this note is urinary bile acid analysis. The synthesis of bile acids from cholesterol is complex and requires modifications of the cholesterol nucleus and oxidation of the cholesterol side chain. Two major metabolic pathways for bile acid synthesis, a "neutral pathway" and an "acidic pathway", and several minor pathways are described; these pathways differ in terms of whether the initial modification is of the cholesterol ring structure or of its side chain. The synthesis of the two normal primary bile acids, cholic acid and chenodeoxycholic acid and their conjugates, involves steps in multiple intracellular compartments and is highly regulated. Several enzymes of bile acid synthesis are common in both major pathways, whereas others are specific for one pathway. Utilization of a particular pathway of bile acid synthesis is tissue-dependent and also depends on an individual's age. The neutral pathway occurs in the liver whereas alternative pathways take place in extrahepatic sites such as brain, lung and macrophages and the acidic pathway is thought to be more important in infancy. Genetic abnormalities are now known for most of the steps of bile acid synthesis.

As is the case for the organic acidurias, genetic disorders causing significant inefficiency or an absence of an enzyme needed for the synthesis of bile acids typically result in a pathologic excess of one or more bile acids in the body. These excess metabolites are excreted in the urine. A pathological excess of abnormal bile acids can also occur when a key enzyme of bile acid metabolism is rendered dysfunctional on a secondary basis such as due to an inhibition of its enzyme activity or due to loss of viable hepatic tissue as occurs in advanced hepatic cirrhosis; this is particularly relevant in the differentiation of primary vs. secondary Δ4-3-oxosteroid 5β- reductase deficiency. Some medications - such as ursodeoxycholate - impact bile acid metabolism. Gut bacterial metabolism of bile acids factors in their enterohepatic circulation and also impacts the pattern of urinary bile acids.

The most common family of disorders screened for by urinary bile acid analysis is the group of inborn errors of bile acid synthesis. Some examples of these disorders include 3-β-hydroxysteroid-Δ5-C25-steroid dehydrogenase deficiency, Δ4-3-oxosteroid 5β-reductase deficiency, sterol 27-hydroxylase

deficiency and oxysterol 7α-hydroxylase deficiency, although there are many others. Urinary bile acid analysis is also an important tool, though not the primary assay, to screen for disorders of peroxisomal biogenesis and peroxisomal beta-oxidation such as Zellweger and neonatal adrenoleukodystrophy syndromes and peroxisomal bifunctional enzyme deficiency.

The clinical presentations and natural histories of the disorders of bile acid synthesis fall in two major categories: gastrointestinal or CNS phenotypes. Most of the genetic disorders of bile acid synthesis present with GI phenotypes. The typical presentation is in infancy with conjugated hyperbilirubinemia and raised serum transaminase levels; other findings can include hepatomegaly, rickets, steatorrhea and coagulopathy from fat soluble vitamin malabsorption. A useful biochemical clue to these disorders is that the conjugated hyperbilirubinemia is not accompanied by increased serum ϒ-glutamyl transpeptidase activity; the deficiency of normal bile acids results in the retention of this enzyme with the canalicular membrane instead of its increase in plasma. If untreated, these disorders usually progress to fulminant hepatic dysfunction.

Several inborn errors of bile acid synthesis have CNS phenotypes. The most recognized form of cerebrotendinous xanthomatosis, a disorder of27-hydroxylase deficiency, presents in childhood or in the adult years. The typical clinical presentation includes spasticity and neuropathy with development of cognitive disability and cataracts, although some forms present in infancy or early childhood with chronic diarrhea or even severe hepatic dysfunction. Several of the few reported cases of oxysterol 7α-hydroxylase deficiency presented with cholestatic jaundice and progressive hepatic dysfunction in infancy whereas others present with 'pure' or 'complex' spastic paraparesis in adulthood. The peroxisomal disorders that can be detected by the screening of urinary bile acids typically present with severe or profound global developmental delays and marked hypotonia, and sometimes have hepatomegaly and craniofacial dysmorphism; hepatic dysfunction is usually not a major part of the clinical phenotype.

From a diagnostic perspective, analysis of urine provides an excellent means for the diagnosis of bile acid biosynthetic disorders. The earliest effective clinical screening assay of urinary bile acids was fast atom bombardment ionization mass spectrometry (FAM-MS). While this method is difficult to automate, it is still in clinical use at some centers and is a very effective tool. Most labs now use electrospray ionization tandem mass spectrometry (ESI-MS/MS). In this method, urine samples, after a clean-up extraction, are injected onto a HPLC column prior to the mass spectrometry. Evaluation of total ion scans and scans of a series of precursor ions is then done; the latter allows for the recognition of glycine-, taurine-, sulfate- and glucuronide-conjugates of the bile acids. The total ion current scan shows the relative amounts of the (presumptively identified) species. Each of the known deficiencies of an enzyme of bile acid synthesis is associated with a characteristic pattern of bile acid species that is readily identified by negative ion mode FAB-MS or ESI-MS/MS and is easily differentiated from common causes of cholestasis. A definitive identification of abnormal bile acids and bile alcohols is usually not needed for most clinical applications

unless there is a large amount of an unusual species or if there is suspicion of a new disorder. In such cases, a definitive identification of the bile acid species can be achieved by a much more laborious GC-MS analysis; other methods are now also available. There are excellent reviews for additional detail regarding the clinical phenotypes and pathophysiology of disorders of bile acid synthesis and for clinical and research methods to evaluate bile acids and related compounds (5-7).

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Pediatric Urinalysis

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Urinalysis in young children and infants is often performed as part of the work-up or diagnosis of urinary tract infections (UTI). UTI is common in febrile children ages 2 to 24 months, with approximately 5% of febrile children in that age range having UTI.[1] Because it is a common finding in febrile infants and children, the American Academy of Pediatrics (AAP) has produced guidelines for diagnosis and management of UTI in children ages 2-24 months.[1] As in adult patients, screening for UTI in children often starts with biochemical and/or microscopic evaluation of urine (urinalysis with microscopic examination of centrifuged or uncentrifuged urine samples, and/or gram stain examination of an uncentrifuged urine sample).

Regarding biochemical tests (generally but not always dipstick urinalysis), leukocyte esterase has the highest sensitivity for UTI (~84%) but relatively low specificity (~77%). Nitrite positivity by conventional dipstick urinalysis has lower sensitivity (~58%) but higher specificity (98-99%).[1] Microscopic detection of > 10 WBC per high power field (HPF) or any bacteria from a sample obtained by suprapubic catheter has sensitivity and specificity of 80-90%.[1] Thus biochemical (usually dipstick) analysis of leukocyte esterase and nitrite, and microscopic examination of centrifuged urine for WBC and bacteria, are the predominant means of screening for UTI in children. The "enhanced urinalysis", consisting of enumeration of WBC using a hemocytometer and gram stain of an uncentrifuged urine specimen, has been demonstrated to be more sensitive and specific than biochemical testing.[1]

While urine culture remains the gold standard for definition of UTI, difficulties in obtaining sterile samples in young children have prompted attempts to define alternatives to culture for UTI diagnosis. A recent systematic review concluded that for many children the combination of a positive leukocyte esterase and nitrite biochemical test; or combination of positive WBC (> 10/HPF) and presence of bacteria can be used to rule in UTI. Similarly, the combination of negative leukocyte esterase and nitrite, or combination of negative WBC and bacterial by microscopy, can be used to rule out UTI in many children.[2] While not explicitly endorsing this concept, the AAP guidelines also mention that given the prevalence of UTI in this population (5%), the probability of a UTI would be reduced to 0.2-0.4% in a child with a negative enhanced urinalysis (<10 WBC/microliter and no bacteria seen on gram stain).[1] In some studies gram stain of a urine sample has been demonstrated to be as or more sensitive than leukocyte esterase and nitrite testing combined. [1] However like culture studies (see below), the gram stain procedure will be more dependent upon the type of urine sample obtained. Practices that use urine gram

stains to screen infants and children under 2-3 years often use only specimens obtained by suprapubic aspiration or catheter.

Urine sample collection from young (recently or not toilet trained) children can be generally classified into four types: suprapubic aspiration, catheter urine, clean catch or assisted clean catch, and bagged urine. Suprapubic aspiration, a minimally invasive procedure involving puncture of the skin to obtain urine from the bladder above the pubis, is considered the "gold standard" for urine collection in infants and small children in that urine obtained in this manner is considered sterile and most appropriate for culture. Ultrasound guidance to insure a full bladder and guide aspiration may increase the success in obtaining a urine sample. Catheter collection is a less invasive (but still often found painful to children) alternative to suprapubic aspiration in young children. The success of clean catch or assisted clean catch collection depends on the age of the child. In some practices assisted clean catch and clean catch collections are the primary means of obtaining urine samples in children older than 3 years of age.

For infants, the alternative (to suprapubic aspiration or catheter collection) is bag collection or use of a similar collection device to obtain urine samples. Bags and similar devices that prevent urine from collecting in diapers have been found to produce "cleaner" samples than gauze pads or other methods to collect urine from diapers. Studies have shown that bag collection improves the value of biochemical urinalysis compared to gauze pads or diaper collection, such that in some instances the biochemical (leukocyte esterase and nitrite) results from a bagged specimen properly collected and tested promptly can be interpreted as would a sample from suprapubic aspiration or catheter collection. However (see below), bag specimens should not be used for urine culture in infants and small children.

For enumeration of bacteria by gram stain or microscopic examination, sample type becomes much more important. Gram stain of an uncentrifuged sample has high sensitivity and specificity (80-90%) when performed on urine collected by suprapubic aspiration or catheterization.[1] Gram stain is less often performed and is of uncertain value in urine samples collected from bag specimens. Reference intervals for WBC (< 5-10 per HPF or microliter) and bacteria (none detected) widely used are most appropriate for samples collected from infants by suprapubic aspiration or catheter.[3]

Automated methods for detection of formed elements in urine (WBC, bacteria, casts, crystals, etc) by image analysis is another common method for performing urinalysis in adults and children. Several studies have been performed to validate and establish reference intervals for WBC and bacteria in uncentrifuged urine samples from children. The studies have differed in age of children (percent not toilet trained), gender, image analysis methods used, and means of urine collection. Reported upper reference intervals for WBC in children vary between 5-50/microliter in most studies; while upper reference intervals for bacteria vary from 3 to >3000/microliter.[4]

Although differences in technology play some role in the large differences in reference intervals, the varied study findings demonstrate the difficulty in determining reference intervals for WBC and bacteria due to differences in collection methods, age and gender. Many laboratories choose not to perform

automated image analysis on pediatric urine samples; in part because of the difficulty in determining reference intervals as a function of technology, collection type, age and gender. In addition the small volume of pediatric urine specimens may not allow for microscopic confirmation of any unusual casts, crystals, or formed elements detected by image analysis.

The gold standard for UTI diagnosis remains urine culture. Regarding sample collection type and urine culture, recommendations are to collect urine samples by suprapubic aspiration or catheter collection for culture in any infant ill enough to require empiric antibiotic treatment.[5] Bag specimens for urine culture are of questionable value, with specificity of bag cultures varying between 14-84%.[1] Assuming a specificity of 70%, the positive predictive value (at 5% prevalence) of a culture from a bag specimen would be only 15%,[1] limiting the utility of cultures from specimens collected in this manner. Therefore many practices rely upon suprapubic or catheter specimens in children under age 2-3 requiring urine culture. For children older than 3 years of age or toilet trained, assisted clean catch or clean catch collection is often used to obtain specimens for gram stain and/or culture.

One final issue regarding biochemical analysis of urine from infants and small children relates to the need perform confirmatory testing on specimens testing positive for glucose by dipstick. Non-glucose carbohydrates will react with the dipstick pad for glucose resulting in a positive test. Galactose (primarily) and other non-glucose carbohydrates may be found in infants and small children with inborn errors of metabolism such as galactosemia. In fact dipstick urinalysis continues to be used as screening tool for galactosemia in under-resourced countries. However all infants born in the U.S. have been screened for galactosemia as part of routine newborn screening for over 10 years. Some laboratory accreditation agencies (e.g. College of American Pathologists) used to require that laboratories have a policy on whether confirmatory testing is performed on urine samples testing dipstick positive for glucose in children under age 2 years. However with expanded newborn screening now routine in developed countries, accreditation agency requirements requiring a policy on glucose dipstick confirmation have largely disappeared. Laboratories should be aware of this issue and consider whether confirmatory testing for positive glucose (by dipstick) results is necessarily based upon the population of patients being served.

Urinalysis in older children and adolescents is primarily done to screen or evaluate children for acute kidney disease, or early onset of chronic kidney disease (CKD). Several countries in Asia require that all school children be screened for early onset of CKD by dipstick urinalysis. A study from Korea summarized the outcome of this screening in approximately 5 million school-age children. Positive results for hemoglobin and protein are common among school-age children. The screening protocol in Korea mandates a second dipstick analysis (both initial and repeat dipsticks using first morning void). If urine samples are repeat positive for blood (heme) or protein, microscopic analysis is performed. Children with microscopic hematuria (> 5-10 RBC/HPF) and/or repeat proteinuria by dipstick are referred to a nephrologist for further investigation. In the Korean cohort, renal biopsy was obtained in 63% of

children referred to a nephrologist for isolated hematuria, 10% referred for isolated proteinuria (a reflection of the high incidence of asymptomatic proteinuria in children), and 70% of children presenting with hematuria and proteinuria. Although the authors did not specify the number of children seen by a nephrologist (the denominator), renal diseases identified included IGA nephropathy (43% of biopsies with a histopathologic abnormality), mesangial proliferative glomerulonephritis (38% of histologically abnormal specimens), and several other uncommon renal diseases. Overall the rate of persistant hematuria was 0.8%, and the rate of persistant proteinuria 0.2%. Children with combined hematuria and proteinuria had a higher rate of renal disease than did children with either isolated hematuria or proteinuria.[6]

In North America and Europe, screening for CKD by dipstick urinalysis is neither recommended nor common. Factors cited in recommendations against routine dipstick screening in children include limited data on cost effectiveness, limited data on the ability to intervene in renal diseases when detected during childhood, and the relatively high prevalence of dipstick proteinuria detected in any single urine exam (up to 10% depending upon age and study) requiring repeat analysis for accurate screening. Use of a first morning void for proteinuria screening (eliminating orthostatic proteinuria) is likely more effective than random screening.[7]

Conclusion

Urinalysis remains a common test in children, primarily for screening and/or diagnosis of UTI in small children. While culture remains the gold standard for UTI diagnosis, studies have shown that a combination of biochemical (leukocyte esterase and nitrite) and/or microscopic (WBC and bacteria) criteria can be used for screening and possibly diagnosis in lower risk children. In higher risk infants and young children for whom empiric antibiotic therapy is being considered, many experts continue to recommend urine culture using a specimen obtained by suprapubic aspiration or cathether. CKD screening of school-age children is currently not widely performed in the U.S, Canada or Europe, primarily due to the uncertain cost vs. benefit of screening based upon dipstick blood (heme) and protein. The combination of persistent hematuria and proteinuria in an older child is more significant than either isolated hematuria or proteinuria.

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EXCERPTS FROM THE LITERATURE

Articles of interest compiled by the editorial board.

A putative role for homocysteine in the pathophysiology of acute bacterial meningitis in children (VLP)

Roney Santos Coimbra, Bruno Frederico Aguilar Calegare, Talitah Michel Sanchez Candiani and Vânia D'Almeida

BMC Clinical Pathology 2014, 14:43

In this article, the authors present data to suggest a role for homocysteine and cysteine measurement in helping diagnose bacterial meningitis (BM). The patient population consisted of 40 children that were admitted under suspicion of meningitis in a period of almost 2 years. The age range was <1 to 13 years old with a median age of 4 years. Of the 40 patients, 9 were confirmed to have bacterial meningitis by CSF culture, 6 infected with pneumococci and 3 with meningococci. Thirteen of the patients were assessed to have viral meningitis based on clinical signs of meningitis but normal CSF protein, glucose, WBC and percentage of polymorphonuclear neutrophils. Homocysteine and cysteine were measured by HPLC. The comparison of laboratory values between the three different populations showed that homocysteine, cysteine, percentage of polymorphonuclear neutrophils, WBC and protein was statistically different between the bacterial meningitis group and the other two populations (normal and viral meningitis.) Homocysteine median values in the BM group was 0.69 μM, and virtually

undetectable in the other groups. Cysteine had a median value of 29.34 μM in the BM cohort and 8.19-8.33 μM in the non-BM patients. The authors further suggest that homocysteine and cysteine are markers of the pathology leading to neuronal death in acute BM. In one patient followed serially, both homocysteine and cysteine decreased as the patient improved. Although the study was comprised of a small sample size in a pediatric population, it does indicate homocysteine and cysteine as promising tools in addition to the current workup in identifying bacterial meningitis. Further studies would be needed to show if this is feasible.

Establishment of hormone reference intervals for infants born < 30 weeks' gestation (JS)

Greaves RF, Zacharin NR, Donath SM, Inder TE, Doyle LW, Hunt RW

Clin Biochem (2014) 47:101-108

Most laboratorians are aware of the difficulties associated with defining reference intervals in a healthy population. Challenges include the large number of participants often required to provide statistical significance and the need to properly define "healthy" depending on the analyte and the population. These challenges are even more complex in the pediatric population, as obtaining access to samples from healthy children isn't always possible. Now imagine trying to obtain large numbers of samples from a healthy population of infants that were born prematurely. Determining the healthy status, obtaining consent, extremely limited sample sizes…this may be the most complicated reference population yet!

Preterm birth is defined as infants of gestational age < 37 weeks at delivery. Prevalence of preterm births varies with geography, but reports indicate 5-18% of all births worldwide fall into this category. Importantly, medical advances have allowed for striking improvements in survival of even the most premature of infants, reaching over 90% survival rates in recent years. Many of these infants will have endocrine measurements performed as they present with hypotension, genital abnormalities and other hormone-dependent issues. Therefore, determining reference intervals in the preterm infant population certainly provides value for clinicians and neonatologists.

It has been reported previously that hormone concentrations are significantly different between extremely preterm infants and older preterm or full term infants. Preterm and full term infants also adapt differently to a world independent of maternal hormones. Therefore, providing age-appropriate reference data is critical to proper interpretation at each of these age intervals. Greaves and colleagues describe a repository of samples from preterm infants used to describe the "normal" intervals for 6 hormones. Due to the dearth of information for this population, this study provides much needed data for the interpretation of hormone concentrations.

As expected, defining the sample population is critical for a study of this nature. Greaves et al. recruited samples over an 18 month period and provide data from 107 infants born from 23 to 30 weeks of gestation. Samples were collected at 1, 4, 7, 14, 21, 28 and 42 days after birth. In order to include only the healthiest of infants, only preterm infants with "the usual presentation with complications specific to prematurity" were considered for participation. Participants were excluded if there were apparent endocrinopathies or congenital abnormalities, metabolic or endocrine disorders as determined by newborn screening tests, or other abnormalities as determined by the neonatologist involved in the study. Any infants that did not survive beyond the equivalent of term were excluded from the final data analysis.

Cortisol, dehydroepiandrosterone sulfate (DHEA-S), growth hormone (GH), progesterone, free thyroxine (fT4), estradiol and insulin-like growth factor-1 (IGF-1) were analyzed. Sample volume is a considerable limitation in a study on the smallest of infants, therefore analytes were specifically chosen to be measured on only one automated chemistry immunoassay analyzer (Siemens Immulite 2000). Intervals could not be determined for IGF-1, as the concentrations in this premature population were lower than the sensitivity of the assay (20 ng/mL). 95% reference intervals were provided for the remaining 6 analytes and were divided between infants born at 23-26 weeks and 27-29 weeks of gestation. The younger group had higher cortisol and DHEA-S and lower fT4 than the older gestational group. Overall, DHEA-S, GH, cortisol and progesterone concentrations were highest in the first week of life and declined thereafter. No significant difference was observed between males and females in the preterm population.

The group also investigated the potential for betamethasone to interfere with measurements performed in this study. Betamethasone is routinely administered to preterm infants to promote lung maturation (92.5% of study participants were exposed to antenatal steroids). Its structural similarity to dexamethasone may lead to assay interference, most notably for cortisol. No association was found between betamethasone or any other perinatal variable investigated and hormone concentrations obtained in this reference interval study. Including this analysis is an important step in confirming the validity of the data collected from such a heterogeneous patient population.

The authors not only report reference intervals for the individual analytes listed, but also provide insight into the anticipated patterns of these hormones in the first six weeks of life. This information may allow for extrapolation of the platform-specific data provided by these authors to overall concentration patterns that could be expected using any method. Providing insight into the values expected in health is critical to the proper diagnosis of disease. In situations of limited data and limited sample size, these insights may be the best we have to aid in diagnosis and interpretation.

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Potential Drug-Drug Interactions are Very Common in Children's Hospitals (UG)

Feinstein, F, Dai D, Zhong W, Freedman J and Feudtner C.

PEDIATRICS (2015), 135; e100-8

Hospitalized pediatric patients are often exposed to an extensive array of distinct medications. It is estimated children with longer stays or rare conditions are exposed to >25 unique medications, increasing their risk of potential drug-drug interactions (PDDIs). In this paper, the authors assessed the prevalence and characteristics of PDDI in pediatric patients treated in children's hospitals. The study used the Pediatric Health Information System (PHIS) database from 43 freestanding children's hospitals and included 1 year data with a total of 498 956 pediatric hospitalizations (patients age <21 years). The majority of patients were aged ≤5 years (51%) and most frequently hospitalizations were due to respiratory diagnoses (19%), 4 days in length (60%) and resulted in home discharge (93%). In these hospitalizations, 49% were associated with ≥1 PDDI, with a "contraindicated" PDDI occurring in 5% of all hospitalizations, a "major" PDDI present in 41%, a "moderate" PDDI in 28%, and a "minor" PDDI in 11%. Top 10 PDDIs stratified by PDDI seriousness are given in the table below. The authors conclude hospitalized patients are commonly exposed to PDDIs, but the subsequent probability of occurrence and magnitude of patient harm requires further empirical substantiation.

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Top 10 Most Frequent Specific PDDIs Stratified According to PDDI Seriousness

Drug–Drug Combination	Potential ADE	Total No. of Exposures	No. of Patients Exposed	Exposure %
Contraindicated				
Ibuprofen and ketorolac	Enhanced gastrointestinal adverse effects (peptic ulcers, gastrointestinal bleeding and/or perforation)	9968	9011	1.81
Fluconazole and ondansetron	An increased risk of QT interval prolongation	17 935	4272	0.86
Calcium chloride and ceftriaxone	Formation of ceftriaxone-calcium precipitates and contraindicated in neonates	4661	3364	0.67
Aspirin and ketorolac	Enhanced gastrointestinal adverse effects (peptic ulcers, gastrointestinal bleeding, and/or perforation)	3095	1639	0.33
Glycopyrrolate and potassium chloride	Risk of gastrointestinal lesions	2583	778	0.16
Calcium gluconate and ceftriaxone	Formation of ceftriaxone-calcium precipitates and contraindicated in neonates	1325	629	0.13
Metoclopramide and promethazine	Increased risk of extrapyramidal effects	1557	596	0.12
Ketorolac and naproxen	Enhanced gastrointestinal adverse effects (peptic ulcers, gastrointestinal bleeding, and/or perforation)	586	524	0.10
Epinephrine and linezolid	Increased hypertensive effects	1010	443	0.09
Atropine and potassium chloride	Risk of gastrointestinal lesions	682	427	0.09
Major				
Fentanyl and morphine	Additive respiratory depression	89 009	65 730	13.17
Fentanyl and midazolam	Additive respiratory depression	114 538	55 824	11.19
Midazolam and morphine	Additive respiratory depression	95 871	45 915	9.20
Bupivacaine and propofol	An increased hypnotic effect of propofol	29 859	28 827	5.78
Lidocaine and propofol	An increased hypnotic effect of propofol	28 293	25 922	5.19
Hydrocodone and morphine	Additive respiratory depression	38 259	25 185	5.04
Morphine and oxycodone	Additive respiratory depression	28 223	18 131	3.63
Lorazepam and morphine	Additive respiratory depression	72 743	16 907	3.39
Fentanyl and lorazepam	Additive respiratory depression	45 469	14 652	2.94
Fentanyl and hydromorphone	Additive respiratory depression	17 911	13 803	2.77
Moderate				
Dexamethasone and rocurorium	Decreased rocurorium effectiveness; prolonged muscle weakness and myopathy	17 822	16 394	3.28
Heparin and vitamin A	Increased risk of bleeding	85 366	10 531	2.11
Propofol and Succinylcholine Chloride	Bradycardia	10 544	10 032	2.01
Midazolan and ranitidine	Increased midazolam bioavailability	21 604	9625	1.93
Midazolam and sevoflurane	Potential of anesthetic effects of sevoflurane	10 067	9212	1.85
Rocuronium and sevoflurane	Enhanced action of rocuronium	8333	7849	1.57
Furosemide and vecuronium	Increased or decreased neuromuscular blockade	20 871	6940	1.39
Dexamethasone and vecuronium	Decreased rocurorium effectiveness; prolonged muscle weakness and myopathy	8117	6698	1.34
Aspirin and furosemide	Decreased diuretic and antihypertensive efficacy	52 952	6633	1.32
Cyclophosphamide and ondansetron	Decreased cyclophosphamide systemic exposure	12 174	6402	1.28

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INTERVIEW WITH A DISTINGUISHED COLLEAGUE: DR. PATTI JONES

BY SHARON GEAGHAN

I had a chance to catch up with Patti via a virtual interview, and she shares her insights as the next in a series of conversations with distinguished colleagues in our discipline. Dr. Jones is President-elect of the AACC.

How did you come to the career decision to choose Clinical Chemistry as your profession?
Definitely through the back door. I took a job in what I thought was a research lab, and ended up running cyclosporine by HPLC on whole blood patient samples from the county hospital. That was my introduction to lab medicine and clinical chemistry.

Did you have a mentor and if so what did he/she teach you?
I've had several mentors along the way, but the one I'd have to say was the most influential for me was Dr. Mike Bennett. He not only taught me everything I know about testing for inborn errors of metabolism, he also got me involved with the AACC. So I'd have to say he taught me to get involved and do my best to make a difference.

For newly-minted chemists, do you have any pearls of wisdom for career development?
Get involved! The AACC is an incomparable place to make contacts and find colleagues and friends. These are people who will help you along your whole career. They are a superb source of knowledge and support.

What is your most enjoyable part of your professional work?
My favorite part is diagnosing inborn errors of metabolism, particularly when we can make a diagnosis of a treatable disorder early enough to make a massive impact on the lives of these kids.

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What is the hardest part of your professional work?
Again, inborn errors. Sometimes we can diagnose when there's no treatment, and sometimes we can tell there's a genetic issue going on but can't yet make a diagnosis, because we don't yet have the knowledge.

The next generation of chemists have been characterized as looking for work-life balance; do you have advice for them, in managing that balance from your experience ?
Work hard while you're at work, and then leave your work at work! Whether you work 6 hour days or 12 hour days, when you leave work, do your best to forget it and focus on non-work. Beyond that, if you're working a job that you really love, it hardly qualifies as "work". It's more of a lifestyle, in itself.

What developments would you most like to see occur in the field, over the next 5 years?
I would like to be able to tell a random stranger what I do for a living and not totally stop the conversation. I'd like him to say, "Oh, yeah. The lab people. You do the blood testing." So within the next 5 years I'd like to see our profession become much more visible to the public.

A "VIRTUAL POSTCARD" FROM ISTANBUL: THE XIIITH INTERNATIONAL CONGRESS OF PEDIATRIC LABORATORY MEDICINE (ICPLM)

BY SHARON GEAGHAN

"Wish you were here"!

The historic, majestic and colorful venue of Istanbul hosted the XIIIth International Congress of Pediatric Laboratory Medicine (ICPLM) on June 20-22nd, attracting over 200 registered participants. The collegial interchange was exceptional for the degree of diversity: scientific representation hailed from more than 40 different countries around the globe. From the Istanbul Congress Center, stunning city views remind the visitor of the amalgamation of cultures, religions and peoples where the continents of Asia and Europe meet.

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The Mission

The mission of the IFCC Task Force on Paediatric Laboratory Medicine is to improve the diagnosis and management of patients from birth to adolescence. The Congress continues a tradition which begun in Jerusalem in 1980, of providing a venue for specialists in pediatric laboratory medicine to meet and exchange scientific work and ideas.

The Congress

The Congress is offered every three years as a three- day satellite meeting, preceding the IFCC WorldLab. Four plenary lectures and twelve symposia offered more than 30 speakers. More than 50 scientific posters in pediatric laboratory medicine were exhibited. Li Wang (BC Children's hospital) and Jakob Zierk (Germany) were poster award winners.

The Organizers

Conference Chair, Professor Vijay Grey, worked tirelessly with Feyza Darendeliler and Űmit TŰrkoŰlu, the IFCC Congress Presidents, to execute an excellent educational program. The Organizing Committee included: Chair Vijay Grey (Canada), Past Chair Klaus Kohse (Germany), Vice-Chair Michael Metz (Australia), and members Tim Lang (UK), Patti Jones (USA), Sharon Geaghan (USA)

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Fortunately, adequate funds were raised from sponsorships and registration fees, for a fiscally sound Congress. The Organizing Committee gives a colossal thank you to the AACC's Pediatric Maternal Fetal Division for its continuous and generous support for this Congress.

Day One: a tour de force from the Turkish Ministry of Health

The Congress opened Friday night with welcoming remarks by Professor Vijay Grey, Chair of the IFCC Taskforce. Dr Bekir Keskinkiliç, Deputy General Director of the Turkish Ministry of Health, gave the first plenary lecture, which was a tour de force covering remarkable progress in Turkey's neonatal screening program. A welcome reception offered an opportunity to reconnect with old friends and make new acquaintances.

Day Two: Dr. Bennett rocks the Congress, and seven symposia focus on the pediatric laboratory

The Congress was kicked off by Michael J. Bennett (Director of the Metabolic Disease Laboratory, Children's Hospital of Philadelphia, USA), who gave the second plenary lecture: Newborn screening for metabolic diseases: saving children's lives and improving outcomes. Then, the first and second symposia focused on neonatal screening and specialized diagnostics for the neonate, respectively. The third and fourth symposia were on nutrition and endocrinology. Next, immunology and allergy testing and pediatric cancers comprised Symposia 6 and 7.

Day Three: A panel on critical values and communications; news on paediatric reference ranges

Wildly popular was a panel discussion on critical values. Global representation provided unique opportunities for discussion of variability in practices and collectively consider what might be best practice.

Dr. Wieland Kiess (University of Leipzig, Germany) gave the third plenary lecture, Metabolic syndrome in childhood and adolescence. Additional offerings on day three included: Symposia 8 and 9, featuring a spectrum of topics unique to Pediatric Laboratory Testing; Symposium 10, abstracts selected for oral presentation; Symposium 11, Educational Opportunities in Pediatric Laboratory Medicine; and Symposium 12, Pediatric Reference Intervals. In this last symposium, speakers from the Swedish national project, the German KiGGs national survey, and the Canadian CALIPER project highlighted progress and new data, along with audience participation.
Founding scientist in the field of pediatric laboratory medicine, Professor Jocelyn Hicks (Washington DC) closed with the fourth and final plenary session on the role of the paediatric laboratory medicine in developing countries and the support required to best serve patients.

A special issue of Clinical Biochemistry comprises the XIIIth International Congress of Paediatric Laboratory Medicine, thanks to Guest Editors, Vijaylaxmi Grey and Klaus P. Kohse, Tim Lang and Michael Metz. The link to this volume (v47 (9):691-864), published June 2014, follows:

http://www.sciencedirect.com/science/journal/00099120

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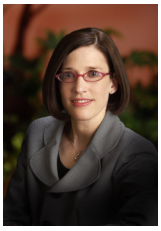
Looking ahead, we are extending an early invitation to join an exceptionally collegial and astute group of pediatric laboratory scientists for the next IFCC Pediatric Task Force Congress, jointly held with the October 2017 IFCC World Congress in Durban, South Africa.

THE CHANGING OF THE GUARD



This edition of the PMF Newsletter will be my last as editor. I have enjoyed putting each issue together, with the help of my fabulous editorial board and contributions from the PMR executive committee and members of the division. But the time has come to hand over the reins, and Joely Straseski, PhD, DABCC, MT (ASCP), FACB, pictured above, has agreed to become the new newsletter editor.
Dr. Straseski is an Assistant Professor and Medical Director at the University of Utah & ARUP Laboratories. I am confident that the newsletter will flourish in her capable hands, and I wish her well.

Angela

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