PEDIATRIC AND MATERNAL-FETAL DIVISION NEWS
Volume 41 | Issue 1 |February 2022

AACC Better health through laboratory medicine.

FROM THE MIND OF THE CHAIR

Happy New Year to everyone! As we welcome in 2022, we have much to look forward to, but still quite a bit to get through. It is my hope that everyone is staying safe and healthy.

Under the heading of things to look forward to, don't forget that abstracts for the 2022 AACC Annual Meeting are due February 24th. If you are eligible for either of the two PMF Division Poster Awards, please check the box to indicate you would like to be considered. Our Division members continue to present outstanding scientific research, and we enjoy rewarding their endeavors.

For those who would like to become more involved in the division, this spring is a golden opportunity. We will be holding elections for the following 5 positions on our Executive Board:

- **Chair-Elect** (includes 2 years as Chair-Elect, 3 years as Chair, and 1 year as Past Chair)
- **Secretary** (a special 2-year term)
- **Treasurer** (3-year term)
- **2 Member-At-Large positions** (each is a 3-year term)

We will also be accepting applications for our Fellow Member of the Executive Board. This is a 1-2 year term for current postdoctoral fellows who have an interest in Pediatric and Maternal-Fetal Clinical Chemistry.

If you have questions about any of these positions, please contact me or any other members of the Executive Board. Keep an eye on our Artery page for more details about the election.

In this issue of the newsletter, we discuss I in The ABCs of Pediatric Laboratory Medicine, and *I is for Icterus*. Excerpts from the literature highlights *Improving Pediatric Urine Drug Testing*. In our Interview with a Distinguished Colleague, we talk with Dr. Mike Astion, our most recent winner of our award for Outstanding Contributions to Pediatric and Maternal-Fetal Clinical Chemistry.

Angela Ferguson, PhD
Chair, AACC PMF Division

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THE ABC'S OF PEDIATRIC LABORATORY MEDICINE:

I is for Icterus: Jaundice in Pediatrics

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Hyperbilirubinemia results from elevated total bilirubin and can result in jaundice, a yellowing of the skin, mucous membranes, and sclera (whites of the eyes). There are diverse causes of hyperbilirubinemia, with some more common in children and others more common in adults [1, 2]. Hyperbilirubinemia in neonates is very common, being present in approximately 60% of full-term infants and 80% of preterm infants [3].

Bilirubin Metabolism

Before discussing the causes of hyperbilirubinemia, it helps to review the biochemical steps of bilirubin production and elimination [4]. Bilirubin is a waste product of heme metabolism. Heme is a component of hemoglobin, myoglobin, and cytochromes. Heme is first converted to biliverdin by heme oxygenase in an unusual chemical reaction that generates carbon monoxide as a byproduct. Biliverdin is then converted to unconjugated (indirect) bilirubin by biliverdin reductase. Unconjugated bilirubin is highly insoluble in water and is mainly transported by albumin in the bloodstream. Although elevated levels of unconjugated bilirubin can manifest as jaundice (and thus may be a sign of pathology), the bilirubin itself usually does not cause direct toxicity. However, extremely high levels of unconjugated bilirubin, especially in infants, can result in kernicterus, a neurotoxicity from high bilirubin concentrations in the brain.

Unconjugated bilirubin is converted to conjugated bilirubin by the enzyme uridine 5'-diphospho-glucuronosyltransferase (UGT) 1A1. In contrast to unconjugated bilirubin, conjugated bilirubin is water-soluble and excreted into the bile for elimination from the body. Bacteria within the gastrointestinal tract break down conjugated bilirubin into compounds such as stercobilin that give stools their characteristic brown color. Conjugated bilirubin cannot cross the blood-brain barrier and thus does not pose a risk of kernicterus.

Assays for Bilirubin

Measurement of bilirubin in body fluids has a long history dating back to 1883, when Paul Ehrlich treated urine with a diazo reagent and found that a red-blue pigment was formed [5]. Over the years, diazo-based bilirubin methods have endured, undergoing periodic modifications. The original diazo methods were found to be most reactive with conjugated bilirubin, even though the exact molecule structure of bilirubin conjugates was not known in the early 20th century. The "direct reaction" of conjugated bilirubin with these early diazo methods led to the naming of "direct" bilirubin assays. Modification of the diazo reactions by adding accelerants (e.g., caffeine, sodium benzoate, methanol) resulted in reactions that measured both conjugated and unconjugated bilirubin [6]. These assays are now termed total bilirubin assays. Since the unconjugated bilirubin fraction could be inferred by subtracting the concentration of direct bilirubin from total bilirubin, unconjugated bilirubin became known as "indirect" bilirubin. The use of two separate assays for measuring total and conjugated bilirubin can occasionally result in the direct bilirubin concentration being "higher" than the total, although usually only by a small amount when concentrations of both total and direct bilirubin are low. Diazo methods have remained as a common analytical methodology for bilirubin measurement on modern clinical chemistry analyzers but can be vulnerable to interferences that absorb in the same wavelength as the azo

products of the reaction. The main alternative method for bilirubin measurement is by vanadate oxidase. One advantage of vanadate oxidase direct bilirubin assays is less interference by hemolysis, a factor that is especially helpful in the neonatal setting where a high proportion of samples may be hemolyzed to some degree [7].

Etiologies of Hyperbilirubinemia

While there are many entities that can result in jaundice, the etiologies of hyperbilirubinemia can be broadly classified into three categories (Table 1) [1, 2]. The first broad category of jaundice results from increased bilirubin production, presenting as a predominantly unconjugated hyperbilirubinemia. The differential diagnosis for this category includes hemolysis, ineffective erythropoiesis, and skeletal muscle damage. In the fetal and newborn period, hemolytic disease of the newborn can result in unconjugated hyperbilirubinemia, with possibility for kernicterus. Other causes of hemolysis in children include infections, transfusion reactions, and glucose-6-phosphate dehydrogenase (G6PD) deficiency. Sickle cell disease and thalassemias may result in both ineffective erythropoiesis and hemolysis. One risk of prolonged unconjugated hyperbilirubinemia is the formation of bilirubin-containing gallstones.

The second broad category of jaundice results from decreased bilirubin conjugation. Similar to increased bilirubin production, this will result in a predominantly unconjugated hyperbilirubinemia. However, the underlying cause relates to insufficient UGT activity relative to bilirubin production. This is a common scenario in the newborn period as UGT enzymes are not fully developed at birth and take approximately 6-12 weeks to reach adult activity levels in full-term infants [3]. As some degree of unconjugated hyperbilirubinemia is common in newborns, this entity is sometimes termed physiologic jaundice of the newborn. The development of UGT enzymes may take even longer in preterm infants, with increased risk of kernicterus in this population. Given the potential risk of unconjugated bilirubin neurotoxicity, the American Academy of Pediatrics recommends universal bilirubin monitoring of all newborns [8].

There are detailed algorithms for the evaluation and management of newborn jaundice. For many neonates, the hyperbilirubinemia will resolve on its own and not need any intervention other than monitoring of bilirubin levels [9].

Table 1. Categories of Jaundice and Causes in Pediatrics

	Broad Categories of Jaundice		
	Increased Bilirubin	**Decreased Bilirubin Conjugation**	**Decreased Excretion of Bilirubin**
Type of hyperbilirubinemia	Unconjugated	Unconjugated	Conjugated
Possible underlying mechanism	• Hemolysis • Ineffective erythropoiesis • Muscle damage	• Immaturity of UGT enzymes (newborn) • Genetic	• Biliary obstruction • Hepatocellular injury • Genetic
Causes in pediatrics	• Infections • Hemolytic disease of the newborn • Sickle cell disease • Thalassemias • G6PD deficiency	• Physiologic jaundice of the newborn • Gilbert's syndrome • Crigler-Najjar syndromes	• Choledochal cysts • Gallstones • Biliary atresia • Bile duct hypoplasia • Alagille syndrome • Viral hepatitis • Epstein-Barr virus • Cystic fibrosis • Galactosemia • Tyrosinemia • Dubin-Johnson syndrome • Rotor syndrome

Nomograms based on serum bilirubin concentrations and postnatal age define risk categories that guide clinical decision-making and treatment. Some infants can be safely discharged from the hospital, with follow-up in the outpatient setting by transcutaneous or serum bilirubin measurements. The first main treatment option for persistent unconjugated hyperbilirubinemia is phototherapy with blue lights, which induces conformational changes in unconjugated bilirubin deposited in the skin. The forms of bilirubin resulting from blue light exposure are water-soluble and can be excreted in bile or urine without need for conjugation. Signs of potentially more serious unconjugated hyperbilirubinemia include jaundice in the first 24 hours of life, jaundice out of proportion with gestational and postnatal age, and bilirubin levels that increase despite phototherapy. These may lead to more aggressive therapy and intensive monitoring. Conjugated hyperbilirubinemia in infants should always be considered pathologic and thoroughly investigated, as this is not an expected part of normal development at any age [3, 10].

In addition to physiologic jaundice of the newborn, two other entities can result in decreased conjugation. Gilbert's Syndrome results from a common genetic mutation in the gene for UGT 1A1. This genetic variant can result in UGT 1A1 activity that is approximately 50-60% that of the average population and may result in mild unconjugated hyperbilirubinemia. Although not pathologic on its own, Gilbert's syndrome can result in decreased conjugation of some medications that are metabolized by UGT. Gilbert's Syndrome is often recognized incidentally by routine laboratory studies. More profound defects in UGT 1A1 occur in the rare Crigler-Najjar Syndromes. Complete absence of UGT 1A1 activity occurs in Crigler-Najjar type 1, a disease which is fatal without blood exchanges and liver transplantation early in life.

The third and last broad category of jaundice occurs due to decreased excretion of bilirubin [2, 10]. The underlying defect here is not enzymatic conjugation but a failure to export conjugated bilirubin into the bile, leading to excess amounts to the bloodstream. This results in a conjugated hyperbilirubinemia with pale stools (lack of stercobilin and other bacterial breakdown products of bilirubin) and brown urine (due to increased urinary excretion of bilirubin). The most common mechanisms of decreased bilirubin excretion are biliary obstruction and hepatocellular injury. Some causes such as pancreatic disease, hepatocellular carcinoma, and metastatic cancer are far more common in adults than children. In biliary obstruction, both conjugated and unconjugated bilirubin may accumulate in serum. Alkaline phosphatase will often be elevated depending on degree of hepatobiliary obstruction. Choledochal cysts (congenital anomaly of bile ducts) and cholelithiasis (gallstones) are the most common diseases causing conjugated hyperbilirubinemia in children. Rare causes include biliary atresia (failure of bile duct development), bile duct hypoplasia (underdevelopment of bile ducts), and Alagille syndrome (multi-organ disorder which may include bile duct paucity or even complete atresia).

There is a wide category of disorders that can lead to hepatocellular injury and conjugated hyperbilirubinemia. In children, causes to consider include viral hepatitis (hepatitis A, B, or C), Epstein-Barr virus infection, cystic fibrosis, galactosemia, tyrosinemia, and autoimmune hepatitis. In general, the presence of conjugated hyperbilirubinemia in children of any age requires work-up and should not be ignored. Lastly, Dubin-Johnson and Rotor Syndrome are rare disorders that can lead to conjugated hyperbilirubinemia due to genetic defects impacting proteins involved in bilirubin transport. These two diseases usually do not cause serious illness.

Icteric Interference

The presence of bilirubin, and potentially also related compounds such as biliverdin, can interfere with laboratory analysis by several mechanisms. The two main mechanisms for how icterus can impact laboratory testing are by spectral interference and chemical reactivity with assay reagents [11, 12]. The spectral properties of bilirubin can interfere with oximetry, co-oximetry, and methemoglobin measurements.

These interferences are related to the absorbance by bilirubin and/or related compounds at specific wavelengths. In terms of chemical reaction interferences, bilirubin is well-known to interfere with peroxidase-coupled reactions used for assays such as triglycerides, total cholesterol, and uric acid. Bilirubin also interferes with Jaffe and enzymatic creatinine methods. Specimen icterus associated with total bilirubin concentrations of less than 10 g/dL usually does not significantly impact clinical laboratory testing. However, the presence of higher levels of icterus is more likely to affect laboratory analysis depending on vulnerabilities of specific assays to icteric interference [13].

Summary

Jaundice with unconjugated hyperbilirubinemia is a common condition in newborns that usually resolves on its own. Monitoring of bilirubin levels in newborns coupled with evidence-based guidelines helps manage more complicated cases. Conjugated hyperbilirubinemia in pediatrics at any age needs investigation, as does unconjugated hyperbilirubinemia occurring outside of the newborn period in the absence of a known cause. Clinical laboratories support clinical care by providing direct and total bilirubin measurements with reasonable turnaround time and by also recognizing potential interference of icterus in patient specimens on certain laboratory tests.

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Excerpts from the Literature

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Improving Pediatric Urine Drug Testing

Life changing decisions hinge on urine drug test (UDT) results in children. From the newborn with increased irritability, the infant with unexplained seizures, to the toddler suspected of ingestion and many other scenarios. Pediatric UDT results directly influence medico-legal interventions and therefore must be as accurate as possible. Despite this, many labs continue to use the 'screen then confirm' approach originally developed for workplace UDT. Although this strategy is practical and cost effective, it is not optimal for pediatric populations. First, the well-known tendency of some immunoassay (IA) antibodies to cross-react with off target compounds leads to false positive screening results. While this phenomenon is accounted for by confirmatory testing, turnaround time can be problematic and provider confusion is common. Second, IA screening is typically performed using cutoffs for positivity that are relatively high. This can lead to false negative results for children that have low level/infrequent exposures and in the context of dilute urine. Finally, the screen then confirm approach is not appropriate for pediatric populations because of the static nature of IA reagents; detectable compounds cannot be adjusted as needed. These drawbacks warrant the use of more contemporary approaches to UDT in children.

Tesfazghi and colleagues article "Development and Implementation of One-Step, Broad-Spectrum, High-Sensitivity Drug Screening by Tandem Mass Spectrometry in a Pediatric Population" investigates the performance of a direct to definitive UDT versus a screen then confirm approach in a large academic hospital setting (1). The authors developed and validated a qualitative LC-MS/MS method targeting a wide variety of compounds with low cutoffs for positivity. This included classic drugs (amphetamines, opioids, benzodiazepines, benzoylecgonine, barbiturates, THC metabolites, PCP etc), as well as less often encountered, but clinically relevant compounds (LSD, ketamine, naloxone, clonidine, gamma hydroxybutyrate, etc). The new method featured a dilute and shoot sample preparation (100 µL urine), 15-minute total run time, and the criteria for positivity were based on empiric data from drug standards. These included relative retention time (vs. internal std), precursor ion mass to charge ratio, the presence of ≥2 characteristic product ions, and the quantitative ratio of the two most abundant product ions. Cutoffs for positivity were determined by serial dilution of drug standards to the lowest concentration that produced a signal to noise ratio of 10. However, some cutoffs were increased based on medical necessity, propensity for carryover, isobaric interference, and/or signal imprecision at the limit of detection. Notably, several parent drug and metabolite pairs were included as targets, including some glucuronidated compounds. This allowed the method to be developed without a hydrolysis step, reducing sample preparation time without sacrificing clinical sensitivity.

The authors evaluated the impact of the new method by calculating rates of positivity and determining the breadth of compounds detected in nursery and general pediatric populations 1 year before and after the method change. Prior to implementing the direct to definitive UDT, the rate of presumptive positivity was 23% in the general pediatric group, with 85% of these going on to be confirmed (by send out LC-MS/MS). IA screening was positive more often (37%) in the nursery population, however the confirmatory rate was much lower (40%). This discrepancy is likely related to the large number of false positive THC IA screens (≈ 97%) in newborns. Following implementation of the LC-MS/MS method, about a third of general pediatric specimens were positive for at least 1 compound compared to 18% of nursery specimens. Regarding variety of compounds detected, cutting out IA testing

doubled the number of drugs identified in the general population. This was not only the result of vastly improved sensitivity for drugs like benzoylecgonine and methamphetamine, but it also reflects positivity for a broader scope of compounds not captured by screening tests at the time (e.g. fentanyl, methylphenidate, clonidine etc). As expected, the turnaround time (TAT) for negative specimens increased with the direct to definitive method. However, a profound improvement in the average TAT for positive specimens was also evident (84 mins vs. 1-3 days) because the assay was setup for random access (24/7) testing (2). Overall, the LC-MS/MS method provided definitive results for a wide variety of compounds in a clinically useful timeframe and has certainly improved care at the authors' hospital.

Considering the consequences of spurious UDT results in children, pediatric labs should move away from the standard screen then confirm approach when possible. Modern LC-MS/MS systems have user-friendly software, resources to assist in method development, and multiple options to ensure high quality, reliable results. With careful consideration of workflows, detailed procedures, and regular oversight, direct to definitive methods can be safe, rapid, and practical for pediatric laboratories.

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Interview with a Distinguished Colleague

By Angela Ferguson, PhD

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What are some challenges you see currently facing the field of laboratory medicine, especially with the current COVID-19 pandemic ongoing?

I think one of the challenges in laboratory medicine is finding enough people to work in labs at all levels. I think this is a long term problem. These are great, meaningful jobs and I hope as a field we can get the word out, and perhaps change our approach to training so we can deploy more people, more quickly.

We could use some more leaders, especially at the doctoral level. I think one area that has promise to partner with business schools to create executive leadership training in laboratory medicine. I think the management training that doctoral level trainees are getting in laboratory management is helpful and necessary but insufficient. The belief that a resident or fellow is equipped to lead a lab right after training is probably not justified in most cases. There is a fair amount of declared expertise in laboratory leadership, by virtue of having spent so long in

training, but actual leadership takes a fair amount of mentoring, practice, and ---most of all—honest feedback. The feedback systems in place for informing people about their leadership problems are inadequate in my view. There is a fair amount of the Kruger-Dunning effect when it comes to lab leadership.

What changes do you see in the future of pediatric or maternal fetal laboratory medicine?

I think that the pandemic will lead to some positive changes. For example:

- there are going to be more treatments for Covid-19 and other respiratory viruses. I think this means that we will continue to do a fair amount of testing, since they will be attached to treatments, analogous to what we have been doing for years for bacterial testing.

- I think there will be more testing at home or near home, and it will take a few forms. For chronically ill kids, such as kids who have received organ transplants, we will see more sample collection at home. Thus, I think there is a good future for dried blood spot collection for monitoring a whole host of analytes in transplant patients, especially immunosuppressive drugs. I also think this same population of chronically ill children might benefit from mobile phlebotomy and other forms of mobile collection. I also think there is some hope for self-collection of respiratory virus specimens and either testing at home or dropping off the specimen at a lab for a rapid turnaround time PCR test.

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