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AACC Better health through laboratory medicine.

FROM THE MIND OF THE CHAIR

Greetings and I hope this message finds you well. To avoid sounding like every new advertising campaign these days, I will just say WOW- this has been quite a spring!

Usually this time of year would be busy with preparations for traveling to Chicago for the 2020 Annual Meeting, and this issue of the newsletter would be full of information about sessions of interest to this group. The ongoing coronavirus pandemic has put a wrench in those plans, along with just about everything else! With the meeting moved to December, please look for that content in an upcoming newsletter.

In this issue of the newsletter, we explore F in The ABCs of Pediatric Laboratory Medicine, and F is for *Fentanyl Exposure in Neonates*. Excerpts from the literature highlights an article on screening for biliary atresia, and the issue closes out with a description of the inflammatory syndrome that can appear in pediatric patients after COVID-19 infection.

This pandemic has shown the vital importance of the laboratory medicine community, and I would like to commend you on all your hard work these past months. Please stay safe and take care, and I look forward to seeing you at the next annual meeting.

Angela Ferguson, PhD
Chair, AACC PMF Division

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THE ABC'S OF PEDIATRIC LABORATORY MEDICINE:

"F" is for Fentanyl Exposure in Neonates

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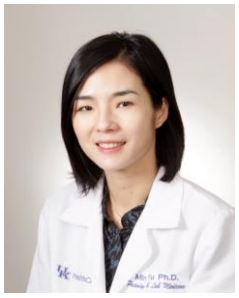

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Fentanyl Epidemic

Fentanyl, a synthetic and rapid-acting opioid, received FDA approval in 1968 as an analgesic for treating severe pain associated with advanced cancer [1]. It is about 100 times more potent than morphine and 50 times more potent than heroin. Due to its extremely addictive potential and growing toxicity issues, fentanyl is listed as a schedule II substance under the Controlled Substances Act [2]. Recently, fentanyl abuse has been recognized as a major driver of opioid overdose deaths starting in 2013. In 2015, Centers for Disease Control (CDC) and the Drug Enforcement Administration (DEA) released nationwide alerts respectively identifying fentanyl as a threat to public health and safety [3, 4]. The CDC reported that from 2011 to 2016, fentanyl-related overdose deaths increased more than 10-fold in the United States [5]. Moreover, the sharp peak in fentanyl overdose deaths and the long-term decline in opioid prescription indicates that the current fentanyl crisis is largely attributed to illicitly manufactured supply, which is usually mixed with heroin and/or cocaine [6, 7]. In addition, the number of fentanyl reports increased from 1041 in 2013 to 56,530 in 2017, according to the data from DEA National Forensic laboratory Information System (NFLIS)[8]. The fentanyl epidemic continues to expand across the United States [9].

Fentanyl Pharmacology

Fentanyl exerts its pharmacological actions predominantly as a full agonist of the μ-opioid receptor [10]. It is therapeutically utilized as an analgesic at low doses (1-2 μg•kg-1) and as an anesthetic at higher doses (>50 μg•kg-1) [11]. In addition to intravenous administration, fentanyl can be administered via multiple non-invasive routes given its high lipid solubility [12]. When delivered via transdermal and transmucosal routes, the bioavailability ranges 50-90% [13]. This highly lipophilic property facilitates quick movement of the molecule from the plasma to its primary site of action in the central nervous system (CNS), with a transfer half-life of 4.7-6.6 minutes [13]. Moreover, it allows fentanyl to readily cross the placenta membrane after administration to a pregnant woman [10].

The pharmacokinetics (PK) of fentanyl are better understood in adults than in children [10]. In adults, fentanyl has a large, dose-independent volume of distribution, and is highly protein-bound (81% at pH 7.4) [14]. It is primarily metabolized by hepatic CYP3A4 to produce norfentanyl, a pharmacologically inactive metabolite [10] [15]. Co-ingestion of fentanyl with certain drugs that interact with CYP3A4 activity may lead to a prolonged fentanyl exposure. The pharmacokinetics of fentanyl are dose-linear [10], and pharmacologic response correlates with plasma concentration [11]. Due to the redistribution

from brain to the poorly perfused fat tissue, fentanyl has a short duration of action of 30-60 minutes after a single IV dose [14]. However, fentanyl stored at those sites can be released back to the circulation, leading to the elimination half-life of 3-8 hours. Moreover, the duration of effect is significantly prolonged with continuous infusion [16]. In adults, fentanyl pharmacodynamics (PD) have been demonstrated to be affected by age; one study found that the dose of fentanyl required to produce EEG slowing decreased 50% from age 20 to 89 [17]

Fentanyl has unique PK in neonates and infants. In neonates, fentanyl clearance is low, which increases risk of drug accumulation [18]. Clearance increases with gestational age [19], increases 3-fold in the first week of life, and continues to increase at a slower rate in subsequent weeks [18]. This may be related to the shift from CYP3A7 expression to CYP3A4 expression in the first week of life [13]. CYP3A4 activity reaches 30-40% of adult activity after 1 month of life [20]. Older infants (3-10 months) have increased clearance (as well as increased volume of distribution) compared to older children and adults [21]. Increased hepatic blood flow (normalized to weight) and/or altered protein binding may account for this difference [10]. Overall, evidence indicates that the inter-individual variation of fentanyl PK is large in neonate and infant populations [22, 23]. Therefore, it is critical to manage each patient individually.

Sources of Fentanyl Exposure in Neonates and Clinical Outcomes

The most common cause of fentanyl exposure in neonates is due to chronic maternal fentanyl usage. Affected by the unprecedented scope of the current opioids crisis described earlier, the incidence of opioid consumption during pregnancy has alarmingly increased. On average, about 33% of childbearing women are prescribed opioids [24] [25]. In addition to increased opioid prescription, the prevalence of non-medical opioid usage or maternal opioid abuse continues to rise. Fentanyl, when consumed by pregnant women for either medical or non-medical use, readily crosses the placenta barrier and reaches the developing fetus. One of the direct negative consequences is the increased risk of the infant developing neonatal abstinence syndrome (NAS) or opioid withdrawal syndrome (NOWS), a complex disorder caused by sudden discontinuation of previously prolonged exposed substances. Clinical presentation of NAS/NOWS involves the CNS, gastrointestinal (GI) system, autonomic system and respiratory system. NAS/NOWS can occur in 42-58% infants born to mothers using illicit opioids [25]. The incidence has increased greater than five-fold from 2004-2014, which is equivalent to one baby born suffered from NAS/NOWS every 15 minutes[26]. The dramatic increase in NAS/NOWS leads to higher neonatal mortality, prolonged length of hospital stay and growing financial burden to healthcare [27]. Clinical manifestations of NAS/NOWS can be influenced by the drugs to which the neonate was exposed, the dosage and the frequency of use, the neonate's metabolism and the associated genetic factors [28]. Although marijuana and methadone were the most studied NAS/NOWS associated opioids, there are increasing reported NAS cases resulting from fentanyl, given its growing popularity [29].

In addition to the prenatal exposure due to chronic maternal usage, infants can be exposed to fentanyl perinatally when it is administered for maternal labor analgesia. With its advantage of fast-absorption and no active metabolites, fentanyl becomes the most common opioid for obstetric use via multiple routes [30]. Of those, epidural is the most widely used, which has been initiated in more than 73% of women in the United States during labor [31]. Regardless of the route of fentanyl administration during labor, it readily crosses the placenta and passes into the fetal circulation, leading to the neonate's exposure to fentanyl. The relationship between neonate safety and maternal fentanyl use during labor has been extensively studied. However, compounded by variables consisting of drugs co-administered,

dosage, duration, patient population and techniques applied, results from those studies are mixed and controversial [29-40]. Common short-term negative infant outcomes include the impaired breastfeeding and neonatal respiratory depression [33, 34]. Furthermore, given the robust evidence indicating a correlation between fetal exposure to other labor medications and a later tendency toward use of drugs of addiction during adulthood, a hypothesis was proposed that the same effect might also apply to fentanyl [41].

Lastly, fentanyl is the most frequently used opioid analgesic in the Neonatal Intensive Care Unit (NICU). As a routine essential practice, it is given to critically ill neonates for pain control and reduction of stress responses. However, it is concerning that the definitive safety of fentanyl has not been well-established [42]. Several studies have revealed that a high rate of opioid tolerance and withdrawal symptoms can occur when continuous fentanyl infusions are administered to critically ill infants [42-50]. The incidence of iatrogenic withdrawal symptoms (IWS) was reported to be up to 50% with infusions lasting longer than 24 hours, and increased to 80-100% with infusions lasting longer than 5 days [44].

Laboratory Evaluation of Fentanyl Exposure in Neonates

Fast and accurate detection of fentanyl is important. While NAS is a clinical diagnosis, drug testing can aid in early identification of affected patients [51]. Neonatal drug testing results also have legal and social implications [51]. Test selection should carefully consider both the specimen and the analytical method.

The most common methods for fentanyl laboratory testing are immunoassay and gas chromatography–mass spectrometry or LC-MS/MS–based methods. Being highly sensitive and specific, mass-spectrometry based methods are currently the gold standard. These methods, based on the mass of fentanyl, can quantify fentanyl concentrations as low as 1–2 ng/mL [52]. Immunoassays that detect fentanyl are available from ARK Diagnostics, Immunalysis Corporation, and Thermo Scientific [52]. Of these, only the ARK Diagnostics platform cross reacts with its metabolite, norfentanyl [52]. Compared to mass spectrometry-based methods , immunoassay methods generally have higher false positive rates [53]. One study found that only 56.7% of specimens that tested positive for opiates by immunoassay could be confirmed by GC-MS [54].

Drug testing to detect in utero drug exposure can be performed on a variety of specimens collected from the mother or the neonate [53], of which meconium is considered the gold standard specimen type [55]. Fentanyl deposits in meconium beginning at ~12 weeks gestation, when fentanyl crosses the placental membrane, is urinated by the fetus into amniotic fluid, and is swallowed by the fetus [56]. This allows for a long window of detection spanning the last ~20 weeks of gestation [57, 58]. This also leads to increased concentrations of drug and high sensitivity compared to other specimen types [53, 59]. However, meconium is not available for every neonate and it may also be contaminated with medication administered to neonates prior to collection.

Umbilical cord tissue is an emerging popular specimen type for neonatal drug testing. An advantage of umbilical cord tissue is that it is reliably available at the time of birth, in contrast to meconium and urine. Cord tissue has a window of detection spanning the third trimester up to delivery [55]. For some opioids, drug concentration in meconium may correlate poorly with detection in cord tissue [55].

Summary

The continuously growing fentanyl crisis and dramatically increasing maternal usage both contribute to an alarming rise in the prevalence of NAS/NOWS, posing a serious social and health concern globally. The PK of fentanyl in neonates is highly variable. Evidences indicate that anesthetic fentanyl exposure can lead to multiple adverse neonatal outcomes. Therefore,

more clinical studies are needed to evaluate the PK-PD relationship and to assess the safety of fentanyl use in these vulnerable patients. Toxicological analysis performed by reliable methods is necessary to help combat the current fentanyl epidemic and improve the health outcomes of infants exposed to fentanyl.

Excerpts from the Literature

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Screening for Biliary Atresia: A Big Impact for Little Patients

The March 24th/31st 2020 edition of the Journal of the American Medical Association (Vol 323 No. 12) includes a study from researchers at Baylor College of Medicine on the diagnostic yield of direct or conjugated bilirubin measurement for biliary atresia (BA) screening (1). The study by Harpavat et al. recognized the impetus for improving the early detection of this life-threatening condition and implemented a prospective 2-stage testing approach to detect BA. In the first stage, more than 120,000 newborns at 14 Texas hospitals were monitored for increased direct or conjugated bilirubin (Bc) using blood collected for routine unconjugated (Bu) hyperbilirubinemia assessment in the first 60 hours of life. Newborns with increased direct or conjugated bilirubin (> 0.2 mg/dL) were selected for stage 2 testing, which entailed a repeat direct or conjugated bilirubin measurement at the two-week well child visit. Infants with repeat testing demonstrating increased levels relative to the stage 1 result, or concentrations >1 mg/dL, were considered positive screens. Individuals with a positive screen underwent additional testing per Hepatology specialists and outcomes were documented to calculate the sensitivity, specificity, and predictive values of the testing strategy.

The first stage of testing yielded a 1.1% positivity rate, whereas about 0.1% (n=119) of the total population tested (n= 123,279) in stage 1 were positive in stage 2. Of the 119 positive screens, 7 patients went on to be diagnosed with BA by intraoperative cholangiogram or pathology assessment of biliary remnant (calculated prevalence of 0.6 per 10,000 births). Of the 112 false positive results, about 50% of these infants had no diagnosis determined, while the other half went on to be diagnosed with non-BA cholestatic disorders, congenital infections, or conditions affecting red blood cell clearance. The group monitored for false negatives by surveillance of enrolled patients seeking treatment at any of the 3 major hepatology centers in the region, although they point out the possibility of missed cases due to follow-up at other locations. As well, they acknowledge cross-reactivity of Bu in direct bilirubin assays as a potential source of the false positives. Overall, the sensitivity of the screening approach was calculated as 100% (95th% CI: 56.1 – 100.0%), specificity of 99.9% (99.9 – 99.9%), PPV of 5.9% (2.6 – 12.2%), and NPV of 100.0% (100% - 100%).

Finally, the study included an assessment of screening impact by evaluating age at Kasai procedure, transplant-free survival, and several other metrics in patients diagnosed with BA before versus during the screening period. Notably, the group that was screened had a shorter time to Hepatology referral, significant decrease in the time to Kasai procedure, and improved transplant-free survival rates.

The study by Harpavat et al. is an outstanding example of the efforts needed to reduce the time to diagnosis of BA in the United

States- an intervention which will improve the quality of many lives. BA, which was considered during the early stages of the Recommended Universal Screening Panel development, meets most Wilson-Junger criteria(2). However, no study has demonstrated reliable measurement of Bc from dried-blood spots and thus proposals for including this disorder in state newborn screening panels have become stagnant. Without a defined process for routine screening, diagnostic workup for BA is typically prompted by prolonged jaundice and/or acholic stools persisting for more than 2 weeks. The resulting delay in disease recognition is tied to poor outcomes for the Kasai procedure and many infants ultimately require liver transplant.

Another factor that may prolong diagnosis is provider desensitization to small increases in direct bilirubin early in life. At institutions where direct/total methods are employed, small increases in direct bilirubin early in life may be written off as Bu cross-reactivity in the context of physiological jaundice(3). A recent CAP neonatal bilirubin proficiency testing participant summary indicates that most labs rely on a derivative of the Jendrassik-Grof method. While these assays are cheap and widely-available, this trend actually reflects the lack of assay options we have for fractionated bilirubin measurement. As laboratory professionals, we may be able to improve the early detection of BA by developing new methods capable of specific Bc measurement. For example, studies evaluating the feasibility of Bc measurement by LC-MS/MS or other methods that are unaffected by a background of Bu could translate into earlier recognition of cholestasis.

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The Clinical Laboratory in Multisystem Inflammatory Syndrome in Children Associated with COVID-19

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Throughout the coronavirus disease 2019 pandemic (COVID-19), perhaps the one bright spot was the low incidence of disease amongst pediatric patients. In the US, pediatric patients have accounted for approximately 4% of total cases, with hospitalization rates well below that of adults.[1] However, in pandemic epicenters in Europe and the US, typically beginning about a month after cases of COVID-19 began to peak, pediatricians observed a marked increase in culture-negative inflammatory diseases. For instance, in Bergamo, Italy, they observed 10 cases of Kawasaki disease in a month.[2] Historically they saw one case of this pediatric vasculitis every 3 months. Meanwhile, across 12 centers in France, in the areas most heavily affected by COVID-19, they reported 35 cases of myocarditis over 2 months.[3] Some of these patients also had features of Kawasaki disease. Two thirds required intubation and mechanical ventilation, most required cardiovascular support with inotropes due to poor heart function, and 10 required extracorporeal membrane oxygenation (ECMO). Ultimately, 6 were found to develop coronary artery dilation,

a complication typically associated with untreated Kawasaki disease. Lastly, in Southern England, as of mid-May, they had seen 38 cases that they felt were consistent with this syndrome.[4] The majority of their patients had shock, and half had myocardial dysfunction. Patients were said to appear similar to Kawasaki disease, toxic shock syndrome, or macrophage activation syndrome. Across all series, most patients had antibodies to SARS-CoV-2. Only a small percentage have had a positive PCR. Thus, this delayed rise of pediatric inflammatory syndrome cases relative to COVID-19 cases as well as positive antibodies and negative PCR in most patients suggested that this is a post-infectious process related to SARS-CoV-2. This pattern has been seen in the US as well in New York[5], Philadelphia[6], and Washington, DC[7].

Fortunately, mortality across all reported series has remained strikingly low. Relative to the incidence of COVID-19 as well as to the population as a whole, incidence of MIS-C is also very low. However, these children often present critically ill or progress to this stage soon after admission. Thus, significant focus has been placed on properly identifying these patients. In response, the US Centers for Disease Control (CDC) released a case definition for what it termed Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with COVID-19.[8] The definition requires that the patient be under the age of 21, have fever, laboratory evidence of inflammation, and at least 2 organ systems involved. Moreover, there can be no alternative plausible diagnosis and there must either be evidence of current or recent SARS-CoV-2 infection or known exposure in the 4 weeks preceding onset of symptoms.

The laboratory workup plays a significant role in the evaluation of these children. For the diagnosis to even be considered, at least one laboratory marker of inflammation must be present, which the CDC defines as an elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, procalcitonin, d-dimer, ferritin, lactic acid dehydrogenase (LDH), or interleukin 6 (IL-6), elevated neutrophils, reduced lymphocytes and low albumin. Most children with MIS-C have more than one of these, but certain labs may also be associated with severity of disease, such as in the setting of particularly elevated ferritin or profound lymphopenia. Additional laboratory testing helps assess organ system involvement. Specifically, troponin or B-type natriuretic peptide (BNP) are often elevated in these children and point to cardiac involvement before an echocardiogram can even be obtained. Acute kidney injury can quickly be assessed for with the blood urea nitrogen and creatinine that are part of the basic metabolic panel. Some children have mild hepatitis that is only detected with a hepatic function panel. Hematologic abnormalities may be seen with the complete blood count or coagulation studies.

Infectious studies are also necessary to ensure the lack of an alternative diagnosis. Some of these studies will be useful for any part of the country, such as polymerase chain reaction (PCR) for adenovirus, enterovirus, Epstein-Barr virus, and cytomegalovirus, depending on the presentation. Others should be dictated by the diseases endemic to that region. For instance, at our institution, the Children's Hospital of Pittsburgh, Ehrlichia and Anaplasma PCRs may be sent for these patients. These tick-borne diseases can be found in western Pennsylvania and may cause presentations that fit many of the clinical criteria above but have very different treatment from MIS-C. Thus, the laboratory workup is also necessary to ensure that the label of MIS-C is not applied incorrectly to prevent delayed appropriate treatment. The clinical description of MIS-C can fit a great number of diseases, and it is only by appropriate laboratory investigations that these other causes can be ruled out. Below demonstrates how we apply this principle, with workup altered by the dominant clinical presentation (Table 1).

Initial Laboratory Work-up	Suspected MIS-C or known KD w/o shock	Shock	Macrophage activation sydnrome or hepatitis
General labs			
CBC	X	X	X
BMP	X	X	X
LFTs	X	X	X
LDH	X	X	X
UA, voided	X	X	X
Lactate		X	X
CRP	X	X	X
PCT	X	X	X
ESR	X	X	X
Ferritin	X	X	X
D-Dimer	X	X	X
Coags with fibrinogen		X	X
CK	X	X	X
Troponin	X	X	X
BNP	X	X	X
ADAMTS13		X	X
Rheumatologic/Immune Labs			
C3		X	X
C4		X	X
Triglycerides		X	X
sIL2r		X	X
Infectious Studies			
SARS-CoV-2 PCR	X	X	X
SARS-CoV-2 serology	X	X	X
Blood culture		X	X
Urine culture if concerning UA		X	X
Respiratory culture (if intubated)		X	X
Adenovirus blood PCR		X	X
Enterovirus blood PCR		X	
EBV PCR and serology			X
CMV PCR and serology			X
HSV blood PCR			X
Ehrlichia PCR (summer/spring)		X	X
Anaplasma PCR (summer/spring)		X	X

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