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The Virtual Autopsy
Will It Make the Scalpel Obsolete?

By Jim Caruso, MD

Every year American dictionaries add new words that have become so common that they are considered mainstream. In the area of medicolegal death investigation, "virtopsy" is trending in that direction. Most of us who work in the areas of forensic medicine and forensic toxicology have heard of the virtual autopsy—an autopsy based more on imaging than physical examination—for which the alias virtopsy is sometimes used. Is the virtual autopsy likely to replace the traditional postmortem examination and eliminate the role of the forensic pathologist in the investigation of sudden, unexpected, violent, or suspicious deaths? The short answer is: not anytime soon.

The use of radiographic imaging as an adjunct in death investigation is not new. It is nearly standard practice to take two-dimensional radiographic images of select cases in most medical examiner and coroner offices. In deaths from gunshot wounds, explosions, fire, and sharp force injuries, the bodies are routinely imaged for the presence of projectiles, shrapnel, or portions of weapons. Some offices frequently image deaths that involve motor vehicles, especially when a pedestrian is involved. And no suspicious or unexplained infant death is complete without the proverbial "babygram."

Radiographic Imaging

Radiographic imaging can also help identify deceased individuals, either by dental means or by comparing antemortem and postmortem images of surgical intervention, implanted devices, or orthopedic hardware. The majority of recently constructed forensic medicine centers in the United States and Canada possess sophisticated imaging capabilities.

Beyond simple two-dimensional radiographic imaging are the modalities of computerized tomography (CT) and magnetic resonance imaging (MRI). For patients with acute medical problems, imaging has become nearly obligatory. In suspected cases of acute appendicitis, a CT scan of the abdomen has all but replaced a thorough but old-fashioned manual examination.

The experimental use of three-dimensional (3D) imaging in forensic pathology evolved naturally in these circumstances. The advantages of an extra axis of view are obvious. A traditional radiograph can aid in recovery of a projectile from deep within the thigh of a deceased individual, but a CT scan can precisely locate what may be an elusive but important piece of evidence.

Multi-slice CT has become an important adjunct in medicolegal death investigations. Current technology allows for approximately 3000 high-resolution images to be put together into a 3D image using special software. One can then gate the images to show specific views, such as skeleton only, or to follow the path of a wound through the body.

Since the early part of the past decade, the body of nearly every deceased service member that has passed through the mortuary at Dover Air Force Base in Delaware has been imaged with a 3D CT scanner. While certainly not essential for every case, these scans have proven their value in the autopsies of thousands of bodies associated with combat and non-combat operations. The autopsy suite in Dover was constructed with a computer screen at each workstation that allows the pathologist to view images at the same time he or she is assessing injuries or looking for metallic foreign objects. This situation is truly ideal.

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Virtual Autopsy

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Rise of the Virtual Autopsy

Some investigators have advocated that sophisticated imaging can entirely replace a traditional autopsy in the same manner that radiographic imaging and sophisticated laboratory testing have somewhat replaced the more traditional history and physical examination in the clinical setting. Those in this camp are relatively few, but a significant number of experts in the medicolegal death investigation field have made the case that 3D imaging could be an important adjunct to the traditional autopsy in select cases. Michael Thali and his colleagues in Switzerland have been pioneers in the field of forensic imaging and prolific contributors to the literature. Thali does not advocate completely replacing the standard autopsy with an imaged virtual autopsy, but does feel that the virtual autopsy can be a screening tool with a traditional autopsy the next step in a subset of cases.

One of the drawbacks of the virtual autopsy is that most forensic cases require a basic level of invasiveness. Toxicological testing is an important part of most forensic autopsies, and retrieving evidence such as projectiles is also essential. Certain natural disease processes are best documented via microscopic examination of tissue sections. Some researchers have attempted to address these issues by incorporating needle biopsies with the imaging studies, and one can easily imagine obtaining toxicological specimens through CT guidance.

Forensic Radiology

This interest in postmortem imaging has spawned the new discipline of forensic radiology. When autopsy suites contained only traditional radiographic imaging equipment, there was little need for the expertise of radiologists. Imaging was used to identify and locate projectiles, document fractures (particularly those of the extremities), and occasionally identify the body. Pathologists are fairly comfortable interpreting two-dimensional images for these purposes. With the introduction of more sophisticated imaging, accurate interpretation became essential. A small group of radiologists took a keen interest in the new discipline of forensic radiology and created texts and formal training programs.

The virtual autopsy has not escaped recognition in the lay literature. For a 2012 article in *Scientific American* titled "Virtues of the Virtual Autopsy," the author interviewed Thali; Ed Mazachowski, the chief deputy medical examiner of the U.S. Armed Forces; and David Fowler, the chief medical examiner of Maryland (1). The interviewees all employ CT scanning in their forensic practices and cite the added value of 3D imaging. None said that the days of the traditional autopsy were numbered.

An article in *The Observer* in 2013 addressed the utility of postmortem imaging in the courtroom (2). This writer also interviewed Thali and discussed an additional capability, the Virtobot. The Virtobot is a mechanized arm that obtains tissue samples under CT guidance. The article cited the advantages of presenting thousands of images as a 3D representation of the decedent to a jury. Fractures and other injuries would remain undisturbed though thoroughly documented. The author said that unless juries became comfortable with being presented virtual autopsy data in place of traditional autopsy findings, defense attorneys would likely exploit the unfamiliarity to their advantage. One of Thali's colleagues, Richard Dirnhofer, compared replacing traditional autopsy findings with those of a virtual autopsy to replacing blood group typing with DNA analysis. He feels acceptance will occur gradually over time.

Utility of the Virtual Autopsy

There are several well-written reviews on the virtual autopsy (3–7). Postmortem 3D imaging has been employed successfully as an adjunct in cases of drowning (8,9), high-velocity gunshot wounds (10), trauma, and suspected elder abuse (11,12). Many medical examiners' offices perform only external examinations on apparent suicides by perforating gunshot wounds or ligature hanging. Postmortem imaging can provide anatomic information that cannot be gleaned from an external examination. A study by Polacco et al. found that virtual autopsy imaging demonstrated cerebral edema, soft tissue injuries from the ligature, and even injuries to the cervical spine and hyoid bone (13). This is far more detail than a simple external examination can provide.

Figure 1 illustrates the information that postmortem imaging can add to a conventional autopsy. In the case of a SCUBA diver who died after making a rapid ascent, CT scans clearly showed a pneumothorax and intracardiac gas, findings that helped a pathologist determine that the cause of death was an air embolism.

Westphal et al. used an interesting combination of imaging modalities—postmortem CT scanning with angiography and a standard autopsy—to provide a 55% increase in diagnostic yield compared with standard autopsy alone in patients with histories of cardiac disease (14). Another group combined postmortem CT with angiography to demonstrate filling defects of the heart and atherosclerosis of the coronary arteries that were not well-appreciated dur-

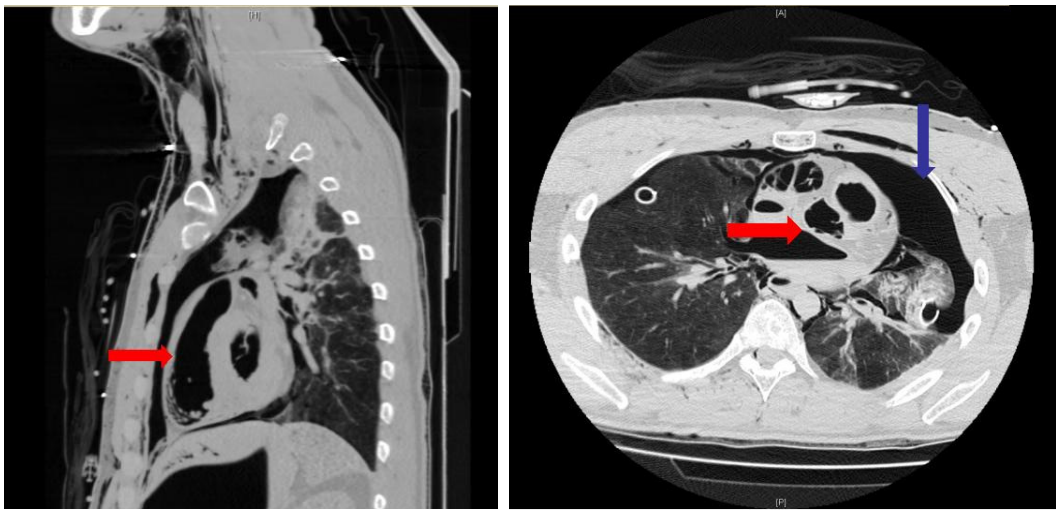

Figure 1. Postmortem Imaging as an Autopsy Aid
CT scans demonstrated that a SCUBA diver sustained a pneumothorax (blue arrow) and air embolism. At left, a sagittal view reveals abundant gas within the cardiac chambers (red arrows). At right, a cross-sectional view shows both the intracardiac gas and the pneumothorax.
Images courtesy of M.J. Pickup, MSc, MD, Ontario Forensic Pathology Service

ing the standard autopsy (15).

Postmortem imaging has also been used to identify bodies and to document trauma in challenging cases, such as charred bodies. These remains may be in such poor condition that imaging studies would be superior to standard dissection (16–18).

The medical examiners at the Dover Air Force Base mortuary have been using multi-slice CT since 2003 and developed considerable expertise. In addition to being able to locate elusive foreign metal objects, such as shrapnel and projectiles, researchers there have used sophisticated software to track gunshot wounds. They can essentially follow the projectile through the body. What sounds like technology far down the line is presently being employed. These medical examiners have the support of radiologists with years of experience in interpreting postmortem imaging. Many of the images from Dover have been used in standard forensic radiology texts (19).

Every medical examiner and coroner has been faced with a family's wishes that an autopsy not be performed. The objections are often based on religious beliefs, but at times the family is concerned about how their loved one might appear at a viewing after an autopsy. Sometimes these objections have no sound basis, but the family remains adamantly opposed to any invasive postmortem examination. To the extent possible, most coroners and medical examiners attempt to abide by the wishes of the next of kin. In homicides and other select cases, an autopsy is generally considered obligatory, despite the preferences of the family. Detailed postmortem imaging may offer an alternative to an invasive examination when the family raises strong resistance (20).

Virtual Autopsy Limitations

Although some studies have touted the superiority of postmortem imaging in detecting traumatic injuries, others have pointed out injuries that were not apparent on imaging but were demonstrated via a traditional autopsy (21). In a series of cases reported by Wichmann et al., traditional autopsy was slightly superior to virtual autopsy in diagnosing cardiac issues and cancer, whereas postmortem imaging was superior in identifying pneumothorax and certain fractures (22). Soft tissue injuries of the spine were better visualized by traditional autopsy than imaging in cases reported by Makino et al. (23).

The sophisticated imaging equipment required certainly limits the virtual autopsy as an option in most medical examiner and coroner facilities. The equipment is expensive and requires persistent maintenance and software updates. Although state-of-the-art CT and MRI units are available at nearly all medical centers, they are used for living patients for whom expenses will be reimbursed. In addition, radiologists with experience in the interpretation of postmortem CT and MRI images are still scarce. The radiologist for whom postmortem imaging is novel tends to overinterpret some postmortem changes as injuries and miss other relevant findings. With experience the discrepancies become few.

Future of the Virtual Autopsy

Postmortem imaging has already proven its value in several areas of forensic medicine. Most new or renovated medical examiner or coroner facilities include at least some sophisticated imaging equipment or can share the CT and MRI assets of a co-located medical facility. As researchers continue to explore new applications for modern imaging modalities in forensic medicine, postmortem imaging is likely to become increasingly mainstream. Radiologists and forensic pathologists will become more comfortable with interpreting these images and differentiating between relevant findings and artifact.

But the virtual autopsy is unlikely to completely replace a standard postmortem examination anytime soon. Rather, the virtopsy will probably solidify its place as an important adjunct in the same manner that

postmortem toxicological testing (the chemical autopsy) and postmortem genetic testing (the molecular autopsy) have done.

Learning Objectives

After completing this article, the reader will have a better understanding of what is meant by a virtual autopsy and how this technology is used in medicolegal death investigation.

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Jim Caruso, MD, is the chief medical examiner and coroner for Denver, Colo. He is a member and former chair of the College of American Pathologists Toxicology Review Committee. Email: James.Caruso@denvergov.org

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Zohydro ER

Medication Offers Extended-Release Hydrocodone without Acetaminophen

By David Kuntz, PhD, DABTF

Pain management is mainly achieved using a variety of opioids. The United States leads the world in their use, with twice as many prescriptions per person for painkillers than second-place Canada. In 2012, healthcare providers wrote 259 million such prescriptions, enough for every American adult to have a bottle of pills (1). Sales of formulations that combine hydrocodone with acetaminophen topped $1 billion and ranked second for all drugs, with 37 million prescriptions dispensed in 2013 (2).

Zohydro ER (Hydrocodone Extended Release) by Zogenix is the first extended-release hydrocodone preparation on the market (3). It joins other single-entity opioids with extended release, including oxymorphone (Opana ER), oxycodone (OxyContin), morphine (Avinza), and hydromorphone (Exalgo).

Zohydro ER is formulated with the drug contained on multilayered beads or spheroids in a system the manufacturer calls the spheroidal oral drug absorption system or SODAS. Each 1–2 mm diameter bead begins as an inert core to which the drug is layered with soluble and insoluble polymers to produce a rate-controlled release.

Zohydro ER is available in six different strengths: 10-mg, 15-mg, 20-mg, 30-mg, 40-mg, and 50-mg capsules. It is designed to provide pain relief for 12 hours rather than the typical 4–6 hours of the standard products with acetaminophen. Products such as Lortab, Vicodin, and Norco contain 2.5–10 mg of hydrocodone and 325–500 mg of acetaminophen per tablet.

Action and Metabolism

Hydrocodone's primary site of action is the mu-opioid receptor, which is responsible for analgesia. Its side effects include sedation, vomiting, respiratory depression, euphoria, anorexia, urinary retention, and physical dependence (3). The delayed-release mechanism results in a longer half-life compared with the combination products, with peak plasma levels occurring about 5 hours after a dose. Steady-state plasma levels are reached after 3 days of dosing. In the manufacturer's dosing studies, steady-state plasma levels for an individual on a 10-mg dose twice daily ranged from 10–15 ng/mL. Steady state plasma levels ranged from 25–30 ng/mL for a 20-mg dose and from 50–70 ng/mL for a 40-mg dose (4).

Hydrocodone undergoes O-demethylation via cytochrome P450 (CYP) 2D6 to hydromorphone and

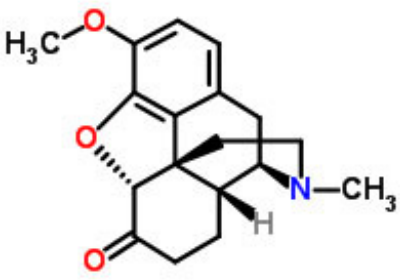

Figure 1. Hydrocodone
Reproduced with permission from www.ChemSpider.com

N-demethylation through CYP3A4 to norhydrocodone. The reduction of the keto group in hydrocodone forms hydrocodol and hydromorphol via phase I biotransformation. In a urine sample collected over 72 hours, 12% of the dose is present as unchanged hydrocodone, 5% as norhydrocodone, 4% as conjugated hydromorphone, and 3% as 6-hydrocodol (5).

Laboratory tests for the extended-release form are the same as existing plasma or urine procedures for hydrocodone, requiring no changes in methods.

Safety Concerns

Although the absence of acetaminophen would seem to make this drug safer, it faced some difficulty receiving Food and Drug Administration (FDA) approval due to concerns about fatalities, addiction, and potential for misuse. There are fears that this single-entity compound could be crushed for snorting or melted for injection to achieve a very fast high, which could be fatal. However, because the drug is bound to polymers in the delayed-release mechanism, this kind of abuse could be difficult.

Zohydro ER was released as a Schedule II drug. Hydrocodone plus acetaminophen products are currently in Schedule III, but the FDA is considering changing them to Schedule II as well.

Zohydro ER is expensive—more than $400 for a 30-day supply. This high cost and concerns about abuse have limited its use, constrained by various state laws and high insurance company co-pays. It is still too early to know whether it will be subject to OxyContin-like abuse.

Learning Objectives

After completing this article, the reader will have a better understanding of the composition of Zohydro ER and its unique sustained release mechanism, hydrocodone metabolism, and safety concerns related to Zohydro's potential for abuse.

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David J. Kuntz, PhD, DABFT, is executive director of analytical toxicology at Clinical Reference Laboratory in Lenexa, Kansas, and a member of the Clinical & Forensic Toxicology News *editorial advisory board. Email: kuntzd@crlcorp.com*

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Drug Tests in Multiple Births

Largest Study Examines Incidence of Mismatches in Meconium Test Results

By Kelly E. Wood, MD, Matthew D. Krasowski, MD, PhD, and Gwendolyn A. McMillin, PhD

Meconium is a common specimen to test for prenatal drug exposure, and positive results can have far-reaching implications for the mother and infant. Reliable test results are critical for making medical and social management decisions, but meconium collection is challenging for a variety of reasons.

Meconium can be unavailable because it was passed during delivery, missed by the collector, intentionally destroyed by the mother, or collected too late, after milk stool began to pass. In most infants, meconium passes within the first two days of life, but it may not pass for three days or more, particularly in premature infants.

Meconium collection is particularly challenging in twins and triplets. Multiple births have an increased chance of premature delivery, and thus longer passage times. Meconium is likely to pass through individuals at different times, be of variable quality between the infants, or be unavailable for one infant.

If meconium is not available from each infant from a multiple birth, is it reasonable to assume that results would be equivalent for all infants? How should results be interpreted when drug-test results are discrepant among twins or triplets?

Studies in Multiple Births

In the United States, the incidences of twins and triplets are 3.3% and 0.1%, respectively (1,2). There has been very limited study of newborn drug screening in multiple births. Most published literature involves twins. Of the positive specimens, most studies reported the same drug(s) in the meconium from both infants, but at different concentrations (3–6).

Until recently, the largest study involved 21 sets of twins and 2 sets of triplets (5). We recently compared meconium drug-testing results for multiple births from a four-year period in a large dataset from a national reference laboratory (ARUP Laboratories, Salt Lake City) and a smaller dataset from an academic medical center (University of Iowa Hospital and Clinics, Iowa City) (7). No clinical information was available for the reference laboratory dataset, which was stripped of patient identifying information. The academic medical center data included medical charts. We analyzed the datasets for mismatched results between twins and triplets and proposed likely explanations for the mismatches.

Testing Protocol

The two-step testing process started with qualitative enzyme-linked immunosorbent assays (ELISA) designed to detect amphetamine, methamphetamine, barbiturates, benzodiazepines, cannabinoid metabolites, cocaine metabolites, methadone, opiates, oxycodone, phencyclidine (PCP), and propoxyphene. All specimens with positive screening results were tested using quantitative gas chromatography/mass spectrometry or liquid chromatography-tandem mass spectrometry assays. Only results that were confirmed by one of these methods were reported as positive.

Results

A total of 2,454 infants in the reference laboratory dataset had meconium analyzed during the study period. Of the 1,084 sets of twins, numbers of female/female pairs, male/female pairs, and male/male pairs were approximately equal. Of the 20 sets of triplets, seven sets were all female, five sets were all male, five sets were male/male/female, and three sets were male/female/female.

In the reference laboratory dataset, 2,168 meconium samples were evaluated by the ELISAs and corresponding confirmatory tests, generating 23,848 results. Cannabinoids and opiates were the two most common drug classes confirmed, with positive rates of 19.3% and 17.3%, respectively. Figure 1 shows the positive rates for each of the 11 drug classes and the percentages of mismatches among siblings.

Mismatched results, defined as qualitatively different results for twins or at least one member of a trio, occurred in two sets of triplets (10%) and 142 sets of twins (13%). The two mismatches in triplets involved opiates in one set and lorazepam in the other. In the twins, the drug classes with the highest

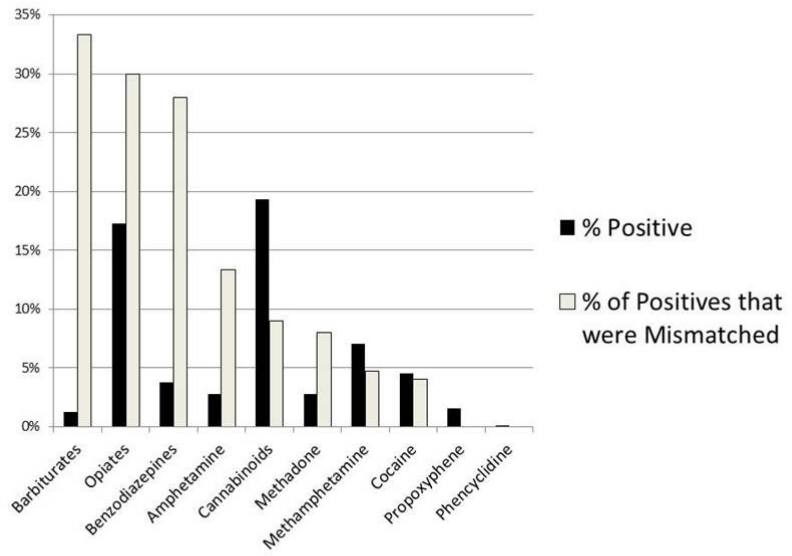

Figure 1. Positive Rates and Mismatches by Drug Class from ARUP Laboratories Dataset
The solid bars indicate the percentage of all samples collected that were confirmed positive. The open bars reflect the percentage of positive samples with mismatched results. The opiates class includes oxcodone.
Figure courtesy of the authors

percentages of positive results with mismatches were prescription drugs, including barbiturates (33%), opiates (30%), and benzodiazepines (28%). No chart review was available for these infants, but based on the Iowa dataset, it is likely that many of these positive results stemmed from medications administered in the hospital.

A possible explanation for these mismatches is that the positive infant received a prescription medication, after birth but prior to meconium collection, but the negative infant did not. Another possible explanation is that a medication was administered to the mother during delivery and one infant passed the meconium immediately after birth, before the drug was incorporated. Tests cannot provide information regarding the time between drug exposure and specimen collection.

Mismatches involving illicit drugs were much less common, and the concentrations were generally very close to the cutoff. This situation suggests that the "negative" twin was probably exposed, but that the concentration of drug in the meconium was below the test's detection limits. There were no mismatches for propoxyphene or PCP, although only one set of twins was positive for PCP.

Iowa Dataset

The University of Iowa dataset contained 109 sets of twins and six sets of triplets.

The only drugs that triggered positive results were opiates (19.5%), benzodiazepines (3.8%), and cannabinoids (2.5%). Mismatched results occurred in two sets of triplets (33%) and 29 sets of twins (27%). Mismatches were most common with benzodiazepines (89%) and opiates (52%). Chart review confirmed that most mismatches (21 of 31) were due to medications administered in the hospital to one infant but not the other, especially lorazepam and morphine.

In five cases of mismatched twin results, there were minor differences in opiate metabolites, but the overall interpretation was the same. In all five cases, both infants tested positive for codeine but showed qualitative differences in possible metabolites of codeine. One case showed codeine alone in one infant, but codeine and morphine in the other. A second case had codeine alone in one infant, but codeine and hydrocodone in the other. The other three cases had more complicated patterns, showing codeine and morphine in both infants, but differences in the presence of hydrocodone and/or hydromorphone between the two infants.

The mothers of all five sets of twins had prescriptions for codeine, and no identified use of other opiates. In all five sets, the difference consisted of detection of morphine, hydrocodone, or hydromorphone barely above the cutoff in one infant but not the other.

There were five mismatches that could not by explained by the patients receiving medications. These all occurred in dichorionic diamniotic twins. Four involved detecting codeine and/or morphine in one twin, but neither drug in the other. The fifth mismatch involved detection of a metabolite of tetrahydrocannabinol in one twin but not the other. The concentration was barely above the lower limit of reporting.

Discussion

Twins and triplets differ in their degree of genetic similarity and sharing of a placenta, which may alter the fetuses' prenatal drug exposure and subsequent testing results. In pregnancies involving twins, both fetuses are theoretically exposed to similar maternal drug levels but drug-testing results may vary. Monozygotic twins share a placenta (monochorionic) (8). Dizygotic twins are genetically different and always have separate placentas (dichorionic). Separate placentas are postulated to explain mismatched

results in drugs and concentrations in dizygotic twins (3,6). In the University of Iowa dataset, all definitive mismatches occurred in dichorionic twins. The three mismatches involving monochorionic twins involved the detection of different opiate metabolites that did not affect the overall interpretation.

Discrepancies in drug-testing results may be related to inconsistent distribution of drug metabolites in the heterogeneous meconium matrix, timing or quality of specimen collection, or specimen mix-up. Mismatches may occur if one infant's sample drug concentration falls below the screening cutoff, but would be detected on confirmatory testing at a lower cutoff value.

In the University of Iowa dataset, differences in prescription medications, particularly benzodiazepines and opiates, administered to one infant but not the other(s) accounted for most mismatches. Mismatches in the national data set, especially phenobarbital, midazolam, lorazepam, and morphine, were likely due to differences in infant medications, but chart review was not available. Such medications are commonly used in the neonatal intensive care unit for sedation, intubation, and pain control.

In premature infants, passage of meconium is more likely to occur later, providing greater time for infant medications to distribute into it (9). Testing of the umbilical cord may overcome these challenges because the specimen would be taken at birth, so drugs administered to the infant would not be an issue. However, the detection window in umbilical cord may be shorter than in meconium (10).

The other most common scenario with mismatched results involved an analyte or analytes barely above cutoff in one infant. It may not have been detected in the other because it was present below the cutoff. This phenomenon was observed frequently with cannabinoid metabolite, for example (7).

Conclusions

Mismatched meconium drug-testing results in twins and triplets are uncommon. Such mismatches are frequently explained by medications administered to one infant but not the other, particularly lorazepam and morphine, in our study. The use of umbilical cord as a specimen would avoid detection of infant medications and perhaps lead to fewer mismatched results.

Separate placentas could be another factor contributing to mismatched results. Low concentrations around the screening test cutoffs could also contribute to only one specimen from a multiple birth testing positive.

Learning Objectives

After completing this article, the reader will be able to describe the challenges in newborn drug testing in multiple births and the common reasons why different results may be seen within a set of twins or triplets.

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Kelly E. Wood, MD, is a clinical assistant professor and director of the newborn nursery in the Stead Family Department of Pediatrics at the University of Iowa Children's Hospital in Iowa City. Email: kelly-wood@uiowa.edu. Matthew D. Krasowski, MD, PhD, is a clinical associate professor and director of clinical laboratories in the depart-

ment of pathology at the University of Iowa Hospitals and Clinics. E-mail: matthew-krasowski@uiowa.edu. Gwendolyn A. McMillin, PhD, is a clinical professor of pathology at the University of Utah School of Medicine and director of toxicology and pharmacogenomics at ARUP Laboratories in Salt Lake City. Email: gwen.mcmillin@aruplab.com.

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