Q&A column

Editor: Frederick L. Kiechle, MD, PhD

Submit your pathology-related question for reply by appropriate medical consultants. CAP TODAY will make every effort to answer all relevant questions. However, those questions that are not of general interest may not receive a reply. For your question to be considered, you must include your name and address; this information will be omitted if your question is published in CAP TODAY.

Q. In a case of suspected drug-related death, how specific can an autopsy be in identifying the drug(s) that might have caused the person's death and the amount of drugs present? For example, can a toxicology report say a person's death was caused by a fake oxycodone pill containing fentanyl?

A.February 2024—An autopsy usually includes toxicology testing, especially in a suspected drug-related death. However, toxicology testing alone is not typically sufficient to determine the cause of death. Toxicology testing, usually performed on blood, first determines whether drugs are present, the identity of the drugs, and the concentrations. These results can help the pathologist determine whether the drugs identified are sufficient to be toxic and account for death. However, it is rarely possible to determine how much of a drug (i.e. the dose) led to the death. Sometimes a single drug may cause death, but often a combination of drugs or alcohol may be responsible.

Toxicology testing alone can rarely tell where the drug came from or how it got into the body (e.g. by mouth, smoking, injection, or other routes). For example, illicit tablets designed to look like pharmaceutical oxycodone tablets have been circulating in North America. Often these tablets contain fentanyl or other opioid painkillers in amounts that may be life threatening to people who lack sufficient opioid tolerance.

More recently, the illicit "fake pills" have been partially superseded by powdered material that may be designed to look like heroin. These powders typically contain little or no heroin, the primary ingredient being fentanyl or another potent opioid (occasionally carfentanil, isotonitazene, or a related opioid). Increasingly, these powders may also contain a sedative such as an illicit benzodiazepine tranquilizer (e.g. etizolam) or the veterinary sedative xylazine. The sedative component of these illicit mixtures is reputed to prolong the "high" of the opioid (e.g. fentanyl). The addition of the sedative component can increase the toxicity of the opioid, which is also primarily a sedative, and that effect is not reversed by naloxone.

In some jurisdictions, drug paraphernalia found at the scene of death (e.g. syringes, powdered drug residue, illicit pills) may be examined, although that is not common in routine death investigations. Unfortunately, it is now common to find multiple drugs in an illicit pill or powder, all of which may have contributed to death to some degree, although in most such mixtures, the opioid is the dominant drug and is primarily responsible for the death.

Ultimately, the final cause of death is determined by an evaluation of the circumstances of death, the autopsy, toxicology testing, medical history, and the scene-of-death investigation. In deaths in which multiple drugs are involved, it is unusual to single out a specific drug as the cause of death because multiple drugs may have contributed to at least some toxicity.

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Q. A nephrology patient who has been treated with vitamin D_2 for several years contacted our laboratory to find out why their 25-hydroxyvitamin D level of 60 ng/mL is now considered elevated when before it was within the normal range. How can we explain this?

A.Vitamin D is a fat-soluble vitamin that regulates calcium and phosphate metabolism and supports bone homeostasis. It is also thought to have a wide range of extraskeletal effects via the nuclear vitamin D receptor, which is expressed throughout the body.

As a preprohormone, vitamin D exists as D_2 (ergocalciferol) and D_3 (cholecalciferol). Ergocalciferol is plant based and can be found in fortified foods, dietary supplements, and prescription form. Cholecalciferol is present in animal-based products and is synthesized in the skin following exposure to ultraviolet B light. Both must be activated through hydroxylation in the liver to generate 25-hydroxyvitamin D (25[OH]D) and in the kidneys to yield 1,25-dihydroxyvitamin D ($1,25[OH]_2D$).

The body stores a significant amount of vitamin D as 25(OH)D, which is the preferred analyte to assess for vitamin D sufficiency (≥20 ng/mL), insufficiency (12 to <20 ng/mL), and deficiency (<12 ng/mL).[1,2] Measuring 25(OH)D is appropriate in populations at high risk of vitamin D deficiency, including people with malabsorption, hyperparathyroidism, osteoporosis, obesity, and limited exposure to sunlight.

Although vitamin D deficiency is a risk factor for a variety of diseases, randomized trials of vitamin D supplementation for primary or secondary prevention of falls, fracture, cancer, cardiovascular disease (including stroke and myocardial infarction), depression, and autoimmune disease have proved unsuccessful.[3,4] Screening of vitamin D levels in asymptomatic adults or during pregnancy is not recommended.[5,6]

The reader's question raises two concerns that need to be addressed. First, the patient asked why their previously normal value is now considered high. Unfortunately, there is no consensus regarding the upper limit of the reference range for 25(OH)D. Some laboratories align the upper limit of the normal range with studies that demonstrate toxicity when serum 25(OH)D levels exceed approximately 80 ng/mL. However, levels above approximately 50 ng/mL are not typically observed in healthy people.

A comprehensive review by the National Academy of Medicine found that almost all people are vitamin D sufficient when serum 25(OH)D levels are greater than 20 ng/mL and that values greater than 50 ng/mL may indicate over supplementation or toxicity.[2] Many laboratories have aligned their reference interval to a range of 20–50 ng/mL. Therefore, it is possible that the patient's laboratory changed its reference range to align with the National Academy of Medicine's recommendations.

However, recommended cutoffs may not be relevant for all laboratories since interlaboratory comparisons have demonstrated that measured serum 25(OH)D levels vary by methodology, assay manufacturer, and instrument, with poor agreement among immunoassays. Immunoassay-measured serum 25(OH)D levels are often inaccurate when compared to levels from liquid chromatography-tandem mass spectrometry-based reference methods.[7,8]

The second concern is that $25(OH)D_2$ does not reliably cross-react with immunoassay detection reagents, leading to under-recovery of $25(OH)D_2$, which contributes to interassay variability. While LC-MS/MS-based 25(OH)D assays can accurately quantify $25(OH)D_2$ and are generally more accurate than immunoassays, the high levels of the C-3 epimer of $25(OH)D_3$ observed in neonates and infants may be insufficiently resolved on chromatography, leading to overestimation of $25(OH)D_3$ levels.[7,8]

Therefore, when testing for vitamin D is warranted, it is critical to understand the limitations of the individual assay and of the reference interval being employed to meaningfully interpret test results and limit clinician and patient confusion.

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