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PEARLS OF LABORATORY MEDICINE

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TITLE: Pain Management: Opioids

PRESENTER: Jada (Yu) Zhang, MD, PhD

Slide 1:

Hello, my name is Jada (Yu) Zhang. I am a clinical chemistry and toxicology fellow at Zuckerberg San Francisco General Hospital, UCSF. Welcome to this Pearl of Laboratory Medicine on "**Pain Management: opioids**" .

Slide 2:

We will go through the introduction of opioids, including opioid family, their biological function, medical application in pain management, side effects, the current opioid crisis, and how to detect opioids in the clinical laboratories.

Slide 3:

The opioid family is defined by their binding capacity to opioid receptors. There are three subgroups, including natural opiates, semi-synthetic opiates, and fully-synthetic opioids.

Natural opiates are natural derivatives from opium, like morphine, codeine, and thebaine. Their chemical structures are quite similar to each other.

Thebaine is proposed as a marker for poppy seed use because it was absent in powdered drugs and the urine of true opiate drug users.

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Semi-synthetic opiates are substituted derivatives of morphine or codeine, including hydrocodone, hydromorphone, oxycodone, oxymorphone, heroin, *etc*. Buprenorphine is a partial agonist, and naloxone is an antagonist, which are used for opioid addiction and overdose treatment, respectively.

Some of the semi-synthetic opiates are also metabolites of codeine and morphine or can metabolize to morphine, which we will get into details in the following few slides.

The structures of semi-synthetic opiates are quite similar to morphine or codeine, with minor differences highlighted with circles. Therefore, many semi-synthetic opiates can cross-react in opiate immunoassays, which target the backbone of morphine. However, their reactivity may be lower. For example, oxycodone and oxymorphone are often missed in urine drug screening by opiate immunoassay due to their lower reactivity.

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Unlike semi-synthetic opiates, fully-synthetic opioids are structurally different from morphine. Therefore, they cannot be recognized by opiate immunoassays, even though they can bind to opioid receptors and perform similar functions. Here are a few examples, including fentanyl, methadone, and tramadol. Methadone is a synthetic opioid agonist that eliminates withdrawal symptoms and relieves drug cravings.

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All the above opioids can bind to opioid receptors and produce analgesia and euphoria. Clinically, opioids have been used for pain management. Codeine, morphine, hydrocodone, hydromorphone, oxycodone, and oxymorphone are the most commonly prescribed opioids for pain management in America.

Naloxone is an antidote to treat opioid overdose.

Buprenorphine and methadone are used to treat opioid addiction. They are a key part of long-term opioid agonist therapy.

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However, opioids do have adverse effects. The opioid-specific toxidrome includes central nervous system and respiratory depression, bradycardia, hypotension, hypothermia, coma, and miosis. Moreover, long-term use of prescription opioids, even as prescribed by a doctor, can develop tolerance, dependence, and addiction. The illicit usage of opioids has caused the opioid crisis in the US.

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From 1999–2018, almost 0.45 million people died from an overdose involving any opioid, including prescription and illicit opioids. In 2018, 2 out of 3 drug overdose deaths involved an opioid. The opioid crisis has imposed a significant social and economic burden in the US. In recent years, fentanyl and synthetic opioids have dominated in the opioid crisis.

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Clinical labs play essential roles in the pain management and opioid crisis. Two main interesting clinical questions include checking patient compliance to prescribed opioids and identify undisclosed recreational drug use.

To answer these questions, *i.e.,* to detect opioids, we need to consider specimen types, analytes, and methods. We will look into the details in the following slides.

The common specimens include urine, blood, and saliva. Each specimen type has its advantages and limitations. The general rule is to examine the earliest specimens available.

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Urine is the most common specimen type for drug tests. Compared to blood, urine usually has a longer detection window, higher concentrations for most drugs, less interference or sample processing. However, it can be tampered intentionally and unintentionally. Therefore, it is important to check the integrity and validity of urine samples. The expected temperature of urine is 90-100 F, and the expected pH is 4.5–

9.0. Dilution or adulteration of urine specimens can be detected by monitoring the creatinine concentration, specific gravity or testing for the presence of oxidants.

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The metabolism of opioids is complicated but crucial for test selection and result interpretation. Opioids are metabolized to active or inactive metabolites through demethylation, glucuronidation, deacetylation, hydroxylation, *etc.* Both parent drugs and metabolites can be excreted into urine. To choose which drug(s) as analytes for drug detection, it depends on the metabolism paths and speed, and clinical needs.

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For most opioids, both parent drugs and primary metabolites are used as analytes to detect drug overdose.

When a patient takes one opioid, both the parent drug and its primary metabolite (s) can be detected. For example, intake of codeine leads to the positive of codeine (the parent drug), morphine, norcodeine, codeine-glucuronide, hydrocodone, and hydromorphone (metabolites).

On the other hand, when one opioid is detected in urine, it may indicate: either the intake of this opioid, or intake of other parent opioids, or sometimes contaminations during drug production. One common example, the detection of morphine may happen when a patient takes morphine, heroin or codeine due to metabolism, or sometimes just due to pharmacologic contamination.

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For some conditions, opioid metabolites, rather than parent drugs, are used as biomarkers of opioid usage.

One condition is to monitor patient compliance with prescribed opioids. Metabolites can exclude the possibility of adding drugs to urine after collection and therefore distinguish real administration from urine adulteration with spiked parent drug only. For example,

EDDP is used to monitor patient compliance when prescribed methadone, and norbuprenorphine for buprenorphine.

The other special condition is drugs with rapid metabolism. For example, heroin metabolizes to 6-MAM with T1/2 of only 2-6 minutes, which makes it less likely to detect parent heroin in urine. 6-MAM and morphine are sequential metabolites of heroin. However, morphine is not unique for heroin use as we mentioned before. Therefore, 6-MAM is a unique biomarker indicating heroin use.

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Here is the list of analytes and detection window for opioids. Opioid use can be detected by the presence of parent drug or its metabolites in urine within 1–4 days. The detection window of 6-MAM is only 12-24 hrs.

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The most common methods to detect opioids are immunoassays and mass spectrometry.

Immunoassays are methods that use an Ag-Ab reaction to detect the drugs. They are usually rapid, easy for automation, and need less labor and cost. However, it is prone to false positive and false negative, and it can't distinguish among opioid(s). Therefore, immunoassays are routinely used for initial opioid screening, and the positive result is only considered as presumptive positive.

Mass spectrometry methods have high specificity, relative high sensitivity, and multiplex capacity. Due to the high demands of instruments, techniques, and cost, MS methods are usually used for opioid confirmation.

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Since opioids are small molecules, **Competitive** immunoassays are applied for opioid detection, including EMIT, CEDIA, FPIA, and Lateral flow immunoassays.

The opiate immunoassays targeting the backbone of morphine can detect natural opiates and many semi-synthetic opiates, but not fully-synthetic opioids. Moreover, they can't distinguish between opioids.

For fully-synthetic opioids and some semi-synthetic opiates, which can't be well recognized by opiate immunoassays, specific immunoassays targeting these drugs should be used. For example, immunoassays for methadone, buprenorphine, oxycodone/oxymorphone, 6-MAM, and fentanyl, are commonly included in the urine drug screening list.

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As you may recall, immunoassays are prone to false positive and false negative. Therefore, positive results can only be considered presumptive positive. Moreover, opioid immunoassays are unable to distinguish between various opioids. Therefore, both positive results, as well as unexplainable negative results, need to be confirmed by MS-based tests.

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Mass Spectrometry is a technique that ionizes, separates, and measures the mass-to-charge ratio (m/z) of molecules for chemical identification and quantification. Compared to immunoassays, MS identifies individual drugs and metabolites by retention time, mass, isotope, MS/MS or transitions, *etc.,* with high specificity and relative high sensitivity.

Different MS methods have been developed for opioids detection. The common methods include gas chromatography (GC)-MS, liquid chromatography tandem MS (LC-MS/MS), liquid chromatography high-resolution MS (LC-HRMS), *etc.*

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The work-flow of mass-spectrometry includes sample purification, molecular separation by chromatography, and molecular identification by mass spectrometry. For each step, there are options according to specimen types, analyte characteristics, and clinical needs.

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Until now, most of MS-based methods are lab-developed tests. For example, SFGH toxicology lab has an LC-MS/MS method for opioid confirmation test, which can confirm and distinguish 13 common opioids, and an LC-HRMS method, which can screen 150 fentanyl analogs and synthetic opioids.

CLSI provides guidelines for method validation of MS methods, including C50-A: Mass Spectrometry in the Clinical Laboratory; C43-A2: 50 Mass Spectrometry in the Clinical Laboratory; C62: Liquid Chromatography-Mass Spectrometry Methods.

Slide 21: Summary

In summary, the opioid family includes natural, semi-synthetic opiates, and fully-synthetic opioids. They have similar biological functions, but fully synthetic opioids are structurally different.

To detect opioids, it is important to select the right specimen at the right time, right analytes, and right methods. Usually, immunoassays are used for screening and MS methods for the confirmation of presumptive positive or unexplained negative results.

Knowledge of the analytical techniques (immunoassays and MS) and how drugs are metabolized are essential to test selection and result interpretation.

Slide 22: References

Slide 23: Disclosures

Slide 24: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Pain management: opioids.”