Article:
Tanner J Freeman, et al.
Clinical Laboratory Detection of a High-Level Hemoglobin Abnormality in a Patient with Suspected Recreational Drug Ingestion.
J Appl Lab Med 2020;5:401-5. https://doi.org/10.1093/jalm/jfz025

Guest: Dr. Terri Jones is an anatomical and clinical pathology third-year resident at the University of Pittsburgh Medical Center in Pittsburgh, Pennsylvania.

Randye Kaye: Hello and welcome to this edition of "JALM Talk," from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye.

Methemoglobin is a special form of hemoglobin that cannot bind oxygen. The blood concentration of methemoglobin in healthy individuals is normally very low, but certain conditions can increase levels of methemoglobin and will result in impaired oxygen delivery to the tissues. These conditions may be congenital or may be induced from the ingestion of specific drugs. Laboratory measurement of methemoglobin is available by CO-oximetry such as on blood gas analyzers and is the preferred diagnostic test for methemoglobinemia. However, a Case Report detailed in the March 2020 issue of JALM describes a spectrophotometric interference that complicated a diagnosis of methemoglobinemia in a patient with suspected recreational drug ingestion.

Our guest for the podcast today is an author from this Case Report. Dr. Terri Jones is an anatomical and clinical pathology third-year resident at the University of Pittsburgh Medical Center in Pittsburgh, Pennsylvania. Dr. Jones is a chief resident and will be starting as a gynecological and breast pathology clinical instructor at UPMC Magee-Women's Hospital in 2021.

Welcome, Dr. Jones. Please begin by telling us about the clinical case that your group recently described in JALM.

Terri Jones: Yes, I would be happy to. So first, I want to credit my co-authors as well for the paper, Tanner Freeman, one of my co-residents; Anthony Scoccimarro, the medical toxicology fellow at our institution; Shauna Henson-Cordwell, the lab technician involved in the case; and Dr. Sarah Wheeler, the medical director of the ATL at the hospital.

So, this all started for the clinical laboratory with a receipt of an arterial blood specimen that looked black and gave error on CO-oximetry. The technologist working that night called the lab's clinical chemist to find out if there is something that

© 2020 American Association for Clinical Chemistry

could be interfering. Consultation with the clinical team indicated that they had empirically determined there was a methemoglobinemia. They had administered methylene blue and shortly thereafter, the patient's blood returned to a more normal deep red hue, but the lab was still left with a question, why had the methemoglobin interfered with the assay?

So going back to our patient, the patient is a 33-year-old African-American male with a history of depression, post-traumatic stress disorder, and alcohol and cocaine use. He was left unresponsive at the emergency department in our institution's hospital system, and on this person were found drug paraphernalia for cooking use, an empty bottle of alcohol, and a bottle of isobutyl nitrate, commonly known as "poppers." His initial vital signs on admission were notable for an elevated pulse of 105 beats per minute and severe hypotension. He clearly had difficulty breathing and his pulse oximetry oxygen saturation of 86% was very low, particularly given that he was on oxygen by nasal cannula.

Administration of naloxone did not help the patient improve and notable labs that were found were acidosis and elevated partial pressure of oxygen and an elevated lactate. His toxicology screen was positive for cocaine, ethanol, and lidocaine, by gas chromatography and mass spectrometry. The patient was intubated and mechanically ventilated in an effort to improve oxygenation.

Upon closer physical examination, the patient had appeared cyanotic. To rule out pulmonary shunt caused by a pulmonary embolism, a CT scan was performed and read as normal. Cyanosis in the absence of pulmonary embolism led to treatment with methylene blue.

Randye Kaye: Thank you. Now, can you fill me in on, what are the clinical symptoms and common causes of methemoglobinemia?

Terri Jones: So to start, let's define methemoglobin. So methemoglobin refers to the ion of a heme moiety being oxidized to the ferric or Fe3+ state which prevents it from binding oxygen. Any remaining ferrous Fe2+ moieties of the hemoglobin tetramer have an increased affinity for oxygen, resulting in a functional anemia.

The physical symptoms in methemoglobinemia occurred to the oxygen deprivation, so his methemoglobin levels rise above 15%, cardiac and neurologic issues such as cyanosis, which is a bluish color of the skin, light-headedness, headache, rapid heart rate and shortness of breath occur.

At higher levels, usually greater than 70% methemoglobin, coma, shock, seizures, and death can occur. Cyanosis becomes clinically detectable when methemoglobin

© 2020 American Association for Clinical Chemistry

comprises approximately 10% of the circulating hemoglobin. However, with our patient, cyanosis may not have been obvious initially given his natural skin pigment. Acquired methemoglobinemia results from exposure to oxidizing substances. There are multiple drugs, both recreationally and therapeutic that can lead to acquired methemoglobinemia such as Dapsone, topical anesthetics like benzocaine and lidocaine, inhaled nitrous oxides, nitrates, and aniline.

So in our patient, the isobutylene nitrate, or poppers, discovered were likely caused of the patient's methemoglobinemia. The patient's urine drug screen was also positive for lidocaine, a common adulterant of drugs of abuse which likely contributed to the methemoglobin production.

Randye Kaye: I see, so in the Case Report, the patient was treated with methylene blue. What is the mechanism of action of methylene blue in the treatment of methemoglobinemia?

Terri Jones: So physiologically, methemoglobin occurs in the range of 0% to 1.5% in the blood healthy individuals. Methemoglobin at these levels can be reduced back to hemoglobin via the function of the enzyme cytochrome b5 reductase which is present on the membranes of red blood cells.

Methemoglobinemia is treated with methylene blue which activates a second methemoglobin reduction pathway that's centered on NADPH methemoglobin reductase which is not normally active.

Methylene blue is reduced by NADPH methemoglobin reductase to leucomethylene blue, which in turn acts as an electron donor to reduce methemoglobin to hemoglobin.

Overall, this reduces the half-life of methemoglobin from hours to minutes and in the case of our patient, he received two 100-mg intravenous doses of methylene blue due to strong clinical suspicion of methemoglobinemia despite not yet having lab results. Within the hour, the patient's pH had actually increased to within normal range and his low blood pressure had resolved.

Randye Kaye: Wow. Now, the Case Report describes unexpected results between the pulse oximetry oxygen saturation measurements and the CO-oximetry results with an interference error code. How do detection methods differ in pulse oximetry versus CO-oximetry, and what are the limitations of each?

Terri Jones: So pulse oximetry, which is routinely used at bedside to monitor a patient's oxygenation, is based on the principle that oxygenated hemoglobin observes greater amounts of infrared

© 2020 American Association for Clinical Chemistry

light as well as lower amounts of visible red light than deoxygenated hemoglobin. The oxygen saturation of the blood is then determined by the ratio of visible red to infrared blood light transmitted.

Methemoglobin absorbs equally well in red and infrared light with a ratio of close to 1. The ratio of 1 translates to an oxygen reading of 85% on the pulse oximeter. This is why our patient's oxygen saturation was around 85% to 90% prior to treatment with methylene blue.

Pulse oximetry is not effective at identifying dyshemoglobinemias such as methemoglobin, carboxyhemoglobin, and sulfhemoglobin, where the hemoglobin species is functionally altered and prevented from carrying oxygen.

CO-oximetry on the other hand, operates on the principle that hemoglobin derivatives have different absorption spectra. Pulse oximeters measure absorption of visible red, as well as infrared light, whereas modern CO-oximeters utilize over a hundred different wavelengths of light. This allows for the identification of more hemoglobin species including dyshemoglobinemias.

The sample spectra generated from the CO-oximeter is then compared to a model spectrum for normal hemoglobin to determine the amount of hemoglobin species present in the sample.

Randye Kaye: Thank you. What do you suspect was the reason for the spectrum mismatch error returned by the CO-oximeter, and what recommendations might you have for any other labs who may come across similar issues?

Terri Jones: When our patient's admission arterial blood sample was run on the blood gas analyzer, the instrument resulted in an OXI-spectrum mismatch error for all measured hemoglobins. The error was suspected to be due to a possible spectrophotometric interference.

Since empiric methylene blue treatment resolved the patient's symptoms, we felt comfortable storing the patient's samples at 4 degrees overnight for follow up the next day. We suspected that either an interfering substance or that the high level of methemoglobin was interfering with the assay, despite the manufacturer's claimed analytical measurement range which was between 0% and 90%. Approximately 12 hours after receiving the initial specimen, the initial sample was rerun neat on the original analyzer and an alternate analyzer which resulted in methemoglobin levels in the range of 56.4% to 61%.

© 2020 American Association for Clinical Chemistry

This may have been due to degradation of an interfering substance or continued reduction of methemoglobin by intraerythrocytic cytochrome b5 reductase. The analyzer may have resulted the OXI-spectrum mismatch error with the patient's admission sample because the difference between the sample and measured spectra, also called the residual spectrum, was too large.

We suspect that overnight reduction of the methemoglobin back to hemoglobin provided a sufficient percentage of reduced hemoglobin to allow for a spectral match and quantification of methemoglobin. Given this, we also expected that the analytical measurement range of our assay was not as high as was reported. So, we utilize these specimens to test for a possible solution. In the clinical laboratory, a one-to-one dilution of blood from the described patient with another patient's remnant blood which had 0% methemoglobin was run on the blood gas analyzer to determine if dilution of a high sample was a feasible method for bringing levels of methemoglobin into the analytical measurement range or diluting possible interference.

The dilution that was performed yielded results within 10% of the neat results after correcting for the dilution. So, we feel that dilution with normal blood is a rapid easy approach to CO-oximeter spectrum errors in a patient with suspected high level methemoglobinemia, which can avoid potential treatment delay. However, we can't rule out degradation during the overnight storage period of another interference that resulted in the initial error readings. But even in these cases, dilution may prove useful to reduce the concentration of the interferant.

Randye Kaye: Very interesting. Dr. Jones, thank you so much for joining us today.

Dr. Terri Jones: Thank you so much.

Randye Kaye: That was Dr. Terri Jones from University of Pittsburgh Medical Center describing a Case Report from the March 2020 issue of JALM entitled "Clinical Laboratory Detection of a High-level Hemoglobin Abnormality in a Patient with Suspected Recreational Drug Ingestion." Thanks for tuning in to this episode of JALM Talk. See you next time and don't forget to submit something for us to talk about.

© 2020 American Association for Clinical Chemistry