Adventures in CO-Oximetry: Apparent Methemoglobinemia

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Clinical History
Patient
A 12-year-old female patient of Southeast Asian origin collapsed while watching a soccer game and was brought to the emergency room of a local hospital by her father.

Chief Complaint
Weakness, syncope, loss of appetite, cough.

History of Present Illness
Prior to hospital admission, the patient was seen as an outpatient at the request of her parents for fatigue, pallor, and lethargy. First menses started 2 months prior. The patient’s family characterized the patient as quiet, a loner, tired, and sleeps as much as possible. Sibling noted the “patient never runs.” The family also noted the patient urinates only twice daily and wets the bed 1 to 2X/week. Urine was foul smelling and turbid. History of constipation with bowel movements 1 to 2X/week.

Past Medical History
Tonsillectomy in 1997; hospitalized for pneumonia in 1995. Patient is reported to be full term vaginal birth with no significant prenatal, perinatal, or postnatal complications. No medication or food allergies are reported. The patient was not on any medications and denied the use of any herbal or natural products as health adjuncts or supplements.

Family History
The family history is negative for diabetes mellitus type 2, coronary artery disease, hypertension, cancers, bleeding disorders, cystic fibrosis, mental retardation, cerebral palsy, sudden infant death syndrome (SIDS), metabolic disorders, glucose-6-phosphate dehydrogenase (G6PD) deficiency, or any other hemoglobinopathies by report.

Social History
The family lives on a farm shared by 3 families. The patient eats a little meat, a hot lunch at school, and dinner cooked by her parents at home. The family obtains drinking water from a common water well. Nonsmoking parents and siblings are healthy with no chronic disorders. The family history is negative for diabetes, hypertension, cancers, bleeding disorders, metabolic disorders, glucose-6-phosphate dehydrogenase (G6PD) deficiency, or any other hemoglobinopathies by report.

Additional Diagnostic Procedures
An outpatient TSH performed a month prior was normal. An echocardiogram was performed and was unremarkable. An ultrasound of the urinary tract revealed a thickened bladder wall with significant bilateral hydronephrosis. A cystometrogram demonstrated adequate overall bladder capacity.

Hospital Course
Following treatment for urinary tract infection, neurogenic bladder, and constipation, the patient was discharged 3 days after admission.

Physical Examination
Based on an elevated methemoglobin (metHb) result, the patient was referred from Hospital A to Hospital B for treatment. The patient appeared pale and tired with some cyanosis around the lips. Vital signs were within normal limits for her age and gender. Respiration rate was 16 breaths per minute and pulse oximetry gave an O2 saturation of 92% on 1.5 L/min oxygen via nasal cannula (about 23% oxygen).

Questions
1. What are the patient’s most striking clinical and laboratory findings?
2. What is the patient’s most likely diagnosis?
3. What is methemoglobinemia (metHb-emia) and its clinical features?
4. What simple observational tests are available to distinguish metHb?
5. What is the treatment for significantly-elevated levels of metHb, and what considerations arise with treatment?
6. What are the causes of acquired metHb-emia?
7. What is the physiologic basis of inherited metHb-emia?
8. What confirmatory tests are available for metHb-emia and dyshemoglobins given positive results by CO-oximetry?
9. What is the significance of the dyshemoglobin abnormality in this patient and outcome?
10. What is the significance of the dyshemoglobin abnormality in this patient and outcome?
11. What is the significance of the dyshemoglobin abnormality in this patient and outcome?
12. What is the significance of the dyshemoglobin abnormality in this patient and outcome?

Possible Answers
1. The patient had a pale cyanotic appearance and a markedly elevated metHb (Table 1) upon admission. C-reactive protein levels suggested an inflammatory process. Urinalysis and urine culture indicated pyelonephritis and treatment was initiated for a urinary tract infection. The most notable finding is the significantly elevated metHb level observed at Hospital Laboratory A as measured by CO-oximetry (Instrumentation Laboratory, GEM OPL, Lexington, MA) prior to transfer of the...
Case Studies

Table 1 Principal Laboratory Findings

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient’s Result</th>
<th>“Normal” Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinalysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>Brown</td>
<td></td>
</tr>
<tr>
<td>Turbidity</td>
<td>3+</td>
<td>None</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.015</td>
<td>1.002–1.030</td>
</tr>
<tr>
<td>pH</td>
<td>8.0</td>
<td>4.6–8.0</td>
</tr>
<tr>
<td>Protein</td>
<td>&gt;100 mg/dL</td>
<td>NEG</td>
</tr>
<tr>
<td>Glucose</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>Ketone</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>Blood</td>
<td>3+</td>
<td>NEG</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>WBC</td>
<td>&gt;100 Cells</td>
<td>NEG</td>
</tr>
<tr>
<td>RBC</td>
<td>&gt;100 Cells</td>
<td>0–2/hpf</td>
</tr>
<tr>
<td>Bacti</td>
<td>4+</td>
<td>None seen</td>
</tr>
<tr>
<td>Esterase</td>
<td>2+</td>
<td>NEG</td>
</tr>
<tr>
<td>Nitrite</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>Clumps of WBCs seen per hpf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine culture: Escherichia coli &gt;100,000 org/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>12.1</td>
<td>4.5–13.5 x 10³/µL</td>
</tr>
<tr>
<td>RBC</td>
<td>3.96</td>
<td>4.0–5.0 x 10³/µL</td>
</tr>
<tr>
<td>Hgb</td>
<td>11.1</td>
<td>12.0–15.0 g/dL</td>
</tr>
<tr>
<td>Pit</td>
<td>416</td>
<td>150–450 x 10³/µL</td>
</tr>
<tr>
<td>MPV</td>
<td>7.6</td>
<td>6.0–9.5 fl</td>
</tr>
<tr>
<td>Neut</td>
<td>71</td>
<td>32–65%</td>
</tr>
<tr>
<td>ANC#</td>
<td>8.6</td>
<td>1.8–8.0 x 10³/µL</td>
</tr>
<tr>
<td>Lymph</td>
<td>15</td>
<td>28–48%</td>
</tr>
<tr>
<td>Lymph%</td>
<td>1.9</td>
<td>1.2–6.0 x 10³/µL</td>
</tr>
<tr>
<td>Mono</td>
<td>12</td>
<td>1–12%</td>
</tr>
<tr>
<td>Chemistry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNP</td>
<td>9</td>
<td>5–100 pg/mL</td>
</tr>
<tr>
<td>D-dimer</td>
<td>0.68</td>
<td>0.10–0.69 µg/mL(DDD)</td>
</tr>
<tr>
<td>Na</td>
<td>140</td>
<td>133–144 mmol/L</td>
</tr>
<tr>
<td>K</td>
<td>3.5</td>
<td>3.4–5.1 mmol/L</td>
</tr>
<tr>
<td>Urea N</td>
<td>13</td>
<td>5–20 mg/dL</td>
</tr>
<tr>
<td>Creat</td>
<td>0.8</td>
<td>0.5–0.9 mg/dL</td>
</tr>
<tr>
<td>Ca</td>
<td>9.1</td>
<td>8.5–10.3 mg/dL</td>
</tr>
<tr>
<td>G6PDH</td>
<td>278</td>
<td>146–376 nU/RBC</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>7.4</td>
<td>0.0–1.0 mg/dL</td>
</tr>
<tr>
<td>Drug screen, urine: none detected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead, blood:</td>
<td>1</td>
<td>0–0.1 µg/dL</td>
</tr>
<tr>
<td>Heavy metals screen, blood</td>
<td>normal</td>
<td></td>
</tr>
<tr>
<td>Arterial blood gas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.45</td>
<td>7.35–7.45</td>
</tr>
<tr>
<td>pCO₂</td>
<td>36</td>
<td>5–45 mm Hg</td>
</tr>
<tr>
<td>pO₂ (room air)</td>
<td>96</td>
<td>80–105 mm Hg</td>
</tr>
<tr>
<td>HCO₃ - calc.</td>
<td>25</td>
<td>22–28 mmol/L</td>
</tr>
<tr>
<td>BE calc.</td>
<td>1.2</td>
<td>-3.0–3.0 mmol/L</td>
</tr>
<tr>
<td>CO-oximetry Hospital Lab A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIO₂</td>
<td>Room Air</td>
<td></td>
</tr>
<tr>
<td>Fractional O₂/Hgb</td>
<td>78.1</td>
<td>94.0–99.0% -Sat</td>
</tr>
<tr>
<td>Carboxy-Hgb</td>
<td>&lt;5.0</td>
<td>0.0–7.0% (nonsmokers &lt;5%)</td>
</tr>
<tr>
<td>Methemoglobin</td>
<td>20.6</td>
<td>0.1–1.9%</td>
</tr>
<tr>
<td>CO-oximetry: Hospital Lab B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methemoglobin: “interferences present”</td>
<td></td>
<td>(Also carboxyhemoglobin, fractional oxymoglobin)</td>
</tr>
<tr>
<td>CO-oximetry: Hospital Lab C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methemoglobin</td>
<td>&gt;10%</td>
<td>0–2%</td>
</tr>
</tbody>
</table>

WBC: white blood cell; RBC: red blood cell; Hgb: hemoglobin; Pit: platelet; MPV: mean cell volume; Neut: neutrophil; ANC: absolute neutrophil count; Lymph: lymphocytes; Mono: monocytes; BNP: brain natriuretic peptide; Urea N: urea nitrogen; Creat: creatinine; G6PDH: glucose-6-phosphate dehydrogenase

patient to Hospital B for further treatment. Following transfer, Hospital B’s laboratory was unable to confirm the elevated metHb levels on an integrated blood gas/CO-oximeter instrument (AVL/Roche, Omni 6, Indianapolis, IN) as identical analyzers gave an “interferences present” printout upon analysis of 3 serial blood samples over a 2-day period for all of the hemoglobin fractions (oxy-, carboxy- and met-hemoglobin). One of the serial samples was transported to a sister hospital 35 miles away (Hospital C) with a standalone CO-oximeter (Instrumentation Laboratory, Model 682, Lexington, MA) that did confirm the original elevated metHb report, giving a level >10%.

2. Most likely diagnosis? Methemoglobinemia. The urinalysis data clearly indicated a urinary tract infection, and the patient was started on an antibiotic, cefixime. Although there were inconsistencies across CO-oximeters, the presence of high levels of methemoglobin on 2 different analyzers plus the patient’s cyanotic appearance supported a diagnosis of metHb-emia.

3. Adult hemoglobin contains 4 polypeptide chains each of which has a heme group with an atom of iron in the ferrous (+2) state which facilitates the binding of oxygen. In metHb-emia, 1 or more of the iron atoms is in the ferric (+3) oxidation state and these heme groups are incapable of binding oxygen. Additionally, the conversion of 1 or more of the iron atoms to the ferric state compromises the overall release of oxygen by the remaining heme groups containing ferrous iron. This causes a functional anemia disproportionate to the metHb level and decreased oxygen release (ie, a leftward shift in the oxyhemoglobin dissociation curve). Normal levels of metHb are generally considered to be <1.5% to 2% with levels of 15% producing an asymptomatic cyanosis. At levels of 20% or more, symptoms include dyspnea, fatigue, nausea, dizziness, headache, and syncope. As levels increase, symptoms worsen, with levels of 70% or more having a high mortality.

4. Deoxyhemoglobin has a dull red appearance, whereas blood containing significant amounts of metHb (and sulfhemoglobin) will have more of a chocolate brown appearance due to significant absorbance at longer wavelengths. Arterial blood from this patient had a brownish appearance consistent with metHb. A convenient bedside test that has been reported is to add several drops of blood to white filter paper. The chocolate brown appearance due to metHb does not change with time, whereas deoxyhemoglobin, which is initially dark red, brightens to a vivid red upon exposure to oxygen due to the formation of oxyhemoglobin.

5. Treatment. The treatment of choice for acutely elevated levels of metHb is methylene blue that is infused intravenously. The dose is usually 1 to 2 mg/kg administered over 3 to 5 minutes and is recommended for metHb levels >20%. Typically, metHb levels will be lowered significantly within an hour. Methylene blue is actually an oxidant, however, and it is the reduced leucomethylene blue form produced in vivo which is the active agent involved in reducing the metHb ferric iron to the ferrous state. This reduced form is generated by a reaction involving the enzyme nicotinamide adenine dinucleotide phosphate (NADPH)-dependent methemoglobin reductase. The NADPH enzyme is produced by the hexose monophosphate shunt pathway and requires adequate levels of G6PDH. Additionally, dextrose infusion is desirable to provide adequate substrate for metabolic conversion of methylene blue to the reduced form. Patients with genetic deficiencies of G6PDH may
not produce sufficient NADPH to convert methylene blue to the active reduced form. In this scenario, methylene blue (being an oxidant) will actually increase metHb levels, and therapy will be ineffective. Exchange transfusion should then be considered as an alternative. A predose G6PDH level in this patient indicated normal enzyme activity (Table 1). Additionally, methylene blue may interfere with CO-oximetry measurements by virtue of its significant absorption in the 600 nm region and weaker absorption at shorter wavelengths. Methylene blue is fairly rapid acting and has a reported half-life of 55 min at normal G6PDH levels. Two doses were administered to the patient during hospitalization with blood metHb levels being sampled 4.5 hours prior to the initial dose and 6.5 hours subsequent to the final dose. Spectral interference by methylene blue in the CO-oximeter measurements was not thought to be contributory to the discordant results among instruments. The metHb level at discharge following 2 cycles of methylene blue treatment and measured at Hospital A’s laboratory was 18.5%.

6. Acquired metHb-emia. Increased metHb levels are frequently a consequence of oxidant exposure. The reader is referred to several recent reviews for a complete listing of drugs and precipitating agents. High nitrate levels in drinking water and certain vegetables has been commonly implicated due to the formation of nitrates produced by endogenous bacteria in the gastrointestinal tract. A number of case studies have also documented the occurrence of local anesthetics such as benzocaine. In the case of this patient, none of these agents or common causes appeared reasonable, given that other family members and other families sharing water or food were unaffected. Subsequent water testing also demonstrated insignificant levels of nitrate. Additionally, no oxidant drugs were detected during the course of a comprehensive drug screen.

7. Inherited metHb-emia. Roughly 3 percent of hemoglobin is converted to metHb on a daily basis. Yet, metHb does not accumulate due largely to the protective effect of cytochrome b5 reductase which facilitates conversion of metHb formed by everyday oxidative processes to functional ferrous-containing hemoglobin. Genetic abnormalities have been reported related to decreased cytochrome b5 reductase (NADH-dependent methemoglobin reductase) activity, which are inherited in an autosomal recessive fashion. A particularly interesting case study of inherited cytochrome b5 reductase deficiency involving several generations of a family from Appalachia has been documented. Additionally, newborns have activities only 50% to 60% of adult levels and are more susceptible to oxidant exposure (eg, with nitrate-contaminated drinking water). Of note is that this red cell enzyme maintains sufficient activity even at refrigerated temperatures and, thus, significantly contributes to the labile nature of metHb in stored samples, resulting in a relatively rapid disappearance of metHb and necessitating its assay in a timely manner. An additional genetic cause of metHb-emia is the presence of hemoglobin M variants. These abnormal hemoglobins have mutations in the globin chain that stabilize heme iron in the ferric state and may also give rise to misleading CO-oximetry results. They can be detected by spectrophotometric scanning of hemolysates, examining absorbances at 500, 600, and 630 nm and calculating absorbance ratios, or by performing hemoglobin electrophoresis. 

8. The lack of an apparent causative agent for metHb in the patient, the lack of agreement across CO-oximeters, and the minimal change in metHb results following 2 doses of methylene blue were problematic in this case. Particularly frustrating was the inability to provide timely data to the clinician following treatment with methylene blue as a consequence of an “interference present” CO-oximeter response at Hospital B where treatment occurred. This was compounded by the observation that the Hospital B instrument was perceived to have more wavelengths available than the other CO-oximeters and ostensibly capable of providing more information. Inquiries to the vendor (Roche) for the AVL/Roche Omni 6 analyzer used at Hospital B indicated that information on the number of wavelengths was considered proprietary, but after further inquiries a service representative indicated that >100 wavelengths are available for resolving hemoglobin fractions in contrast to only 7 wavelengths for the instruments used at Hospitals A and C. Because of these inconsistencies, consideration was given to other means for assessing the presence of metHb. In particular, a relatively simple manual method for metHb is available in standard textbooks. This assay involves measuring metHb at its peak absorbance of 630 nm and involves the addition of cyanide to convert metHb to cyan-metHb, which absorbs at shorter wavelengths resulting in an absorbance decrease at 630 nm due to disappearance of metHb. Given the additional availability of a scanning spectrophotometer, such a procedure was initiated as an ad hoc confirmatory test. Results from this experiment were remarkable in that no reactivity or absorbance change was observed at 630 nm upon addition of cyanide. Additionally, upon scanning, an absorbance peak was observed at 620 nm rather than at the stated metHb peak of 630 nm. This 620 nm absorption peak is consistent with the presence of sulfHb rather than metHb. Reanalysis of the same sample 24 hours later after overnight refrigeration again showed a 620 nm peak with a comparable absorbance, a finding also inconsistent with the labile nature of metHb. Based on these observations, blood was referred on the final day of discharge for a “methemoglobinemia evaluation” (Mayo Medical Laboratories) for a reflexive panel of tests involving hemoglobin electrophoresis, metHb, and sulfHb quantitation, methemoglobin (cytochrome b5) reductase activity, and a hemoglobin spectral scan providing the absorbance ratios A<sub>630/600</sub> and A<sub>500/600</sub> for detection of hemoglobin M variants. Results received several days later are summarized in Table 2 and confirmed the qualitative observations in the authors’

<table>
<thead>
<tr>
<th>Day of discharge</th>
<th>Patient’s Result</th>
<th>“Normal” Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin A2</td>
<td>3.1</td>
<td>2.0–3.3%</td>
</tr>
<tr>
<td>Hemoglobin F</td>
<td>2.2</td>
<td>0.0–2.0%</td>
</tr>
<tr>
<td>Variants:</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>Methemoglobin</td>
<td>0.0</td>
<td>0.0–1.5%</td>
</tr>
<tr>
<td>Sulfhemoglobin</td>
<td>5.3</td>
<td>0.0–1.0%</td>
</tr>
<tr>
<td>Methemoglobin</td>
<td>1.43</td>
<td>≥1.25</td>
</tr>
<tr>
<td>M 630/600 nm ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methemoglobin</td>
<td>3.25</td>
<td>≥2.80</td>
</tr>
<tr>
<td>M 500/600 nm ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methemoglobin reductase</td>
<td>14.1</td>
<td>8.2–19.2 IU/g Hb</td>
</tr>
</tbody>
</table>

Small abnormal peaks on HPLC and spectral scan consistent with sulfhemoglobin. Slight increase of Hgb F of uncertain significance.

3 weeks later:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>“Normal” Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfhemoglobin</td>
<td>2.5</td>
<td>0.0–1.0%</td>
</tr>
</tbody>
</table>
laboratory obtained during admission regarding the presence of sulfHb rather than metHb.

9. SulfHb is a green-pigmented molecule with a sulfur atom incorporated into the porphyrin ring of heme. SulfHb is thought to be derived from hydrogen sulfide produced by intestinal bacteria. Increases have been associated with drug exposure, occupational exposure to sulfur compounds, and environmental exposure to polluted air. A common, although not universal, finding has been a history of chronic constipation. A 1950s study of 62 cases of sulfhemoglobinemia at the Mayo Clinic noted this symptom in 26 patients.

10. SulfHb also causes a functional anemia in that the altered heme-like metHb is incapable of transporting oxygen. Whereas metHb causes a leftward shift in the oxyhemoglobin dissociation curve resulting in a decreased oxygen release at the tissue level, sulfHb causes a rightward shift. Thus, while the patient with sulfHbemia would appear bluer than the patient with a comparable level of metHb due to spectral differences between the pigments, the patient with sulfHb would have less of a tissue oxygenation deficit because oxygen delivery is facilitated rather than impaired.7 SulfHb is also resistant to treatment with methylene blue and is eliminated with normal red blood cell turnover.

11. A sulfhemoglobin result of 5.3% was obtained, which, based on the admission hemoglobin of 11.1 g/dL, would give a sulfHb concentration of 0.6 g/dL. Levels of 0.5 g/dL or more are reported to produce a skin discoloration equivalent to that for deoxyhemoglobin levels of 5 g/dL or more (ie, a discernible cyanosis).9 This level of sulfHb, however, would not likely be expected to cause chronic fatigue and dizziness. Thus, the patient’s fatigue is more likely a consequence of her urinary tract infection. The cause of the increased sulfHb level is also unclear, although there is a strong association with chronic constipation.8 With counseling by medical staff, patient bowel habits were improved, and the quantitative sulfHb level decreased about half-fold to 2.5% (Table 2) several weeks later.

12. Lessons learned. Three current-generation CO-oximeters failed to identify the hemoglobin abnormality present in this case with 2 of the instruments giving erroneous levels for metHb. Documentation for 2 of the 3 instruments specifies the ability to detect sulfHb, but neither instrument provided any indication in this case. A false elevation of metHb in the presence of sulfHb had been reported previously using an earlier-generation CO-oximeter. Identification of sulfHb was likewise confirmed by manually scanning both before and after the addition of neutralized cyanide solution. In vitro production of metHb was also noted following freezing of samples and use of fluoride-oxalate collection tubes.10 Seemingly, identification of dyshemoglobins such as sulfHb in the presence of other blood pigments (eg, lipemia and bilirubin) still remains a challenging task even with current-generation analyzers and appropriate sample collections. Instruments which incorporate diode array technology and which enable sampling at 100 or more wavelengths would appear to have advantages, but this is also dependent on robust computer algorithms for identifying dyshemoglobins and discriminating against other artifacts and interferences. The availability of a recording spectrophotometer for scanning a hemolysate was particularly valuable in this situation and provided an important clue. Additionally, rapid transport of samples to laboratories over a distance of 35 to 50 miles, and the cooperation of staff in providing results using different analyzers was helpful in creating awareness of a problem. Fortunately, the treatment for metHb-emia is relatively innocuous (given normal G6PDH activity) and did not exacerbate clinical outcome. Use of a manual metHb method and, in particular, reactivity (or lack of reactivity) of the hemolysate with cyanide was useful in resolving this case, although recognition was not timely enough to prevent treatment for metHb-emia. IM

Keywords: CO-oximetry, methemoglobin, sulfhemoglobin, dyshemoglobins

4. Trost C. Blue People of Troublesome Creek. Science. 82 Nov. 1982. Available at: www.nclark.net/BluePeopleofTroublesomeCreek.html.