ILLUSTRATIVE CASE

Sometimes, It’s Just Black and White: Dark Urine and Pallor in a 2-Year-Old Boy

Benjamin Wadowski, BS, a Denis Chang, MD, b Sabina Q. Khan, MD, b Tanya Chadha, MD a,c

A 2-year-old Chinese boy was admitted to the hospital after 2 days of pallor and fatigue and 1 day of dark urine. There was no recent history of illness, although his mother reported a 1-week history of intermittent, subjective fevers. There was no history of recent travel, no history of recent trauma, no sick contacts, no pertinent family history, no changes in diet, and no medications. His past medical history was significant for self-limited nosebleeds, which had increased in frequency over the few days before admission.

On examination, the patient was afebrile and tachycardic, with an oxygen saturation of 88% on room air. He was pale and tired-appearing on arrival. He was in no respiratory distress, and there was no hepatosplenomegaly or lymphadenopathy. His skin examination was notable for jaundice, but there were no bruises, petechiae, or rash. He was noted to pass cola-colored urine while in the hospital. His chest radiograph was normal. Laboratory evaluation revealed a normal serum electrolyte level and normal serum urea nitrogen and creatinine levels. A complete blood count (CBC) revealed the following: a white blood cell count of 20,700 per μL with normal differential, hemoglobin of 4.1 g/dL, hematocrit of 11.8%, a platelet count of 365,000 per μL, a mean corpuscular volume of 88.2 femtoliters, and a red cell distribution width of 20.5%.

Question What Are the Current Recommendations for the Evaluation of Anemia in the Pediatric Population?

Discussion

Anemia is estimated to affect up to 20% of the American population during childhood. It can be defined on the basis of age- and sex-specific percentile criteria for hemoglobin concentration. The Centers for Disease Control and Prevention, for example, uses the fifth percentile and below to define anemia, resulting in a lower limit of normal range of 11.0 to 11.9 g/dL for children under age 12 and 12.5 to 13.5 g/dL for children ages 12 to 18 years. Reference ranges for neonates vary considerably on the basis of factors such as gestational age and day of life, although standardized reference values have been established. The diagnostic approach to anemia combines a thorough history and physical examination with laboratory testing aimed at identifying which cell lines are affected, characterizing the patient’s erythrocytes, and differentiating between aplastic and consumptive processes.

The CBC, which is used to confirm the presence of anemia, will also reveal whether the patient has abnormalities in the leukocyte or thrombocyte lines. Several acquired or inherited diseases can contribute to peripheral cytopenia, and many,
but not all, are hematologic in nature. Although a number of consumptive and autoimmune hemolytic processes can cause both anemia and thrombocytopenia, deficiency in all 3 lines suggests a more central disorder of cell production and raises suspicion for aplastic or oncologic processes. These can be further subdivided into inherited versus acquired bone marrow failures. In contrast, anemia can also be coupled with elevation in thrombocyte or leukocyte counts.

Thrombocytosis is an acute-phase reaction and can be found alongside anemia in conditions such as iron deficiency and infection. Although white blood cells are also acute-phase reactants, leukocytosis along with anemia may signify either infection or an oncologic disease and should be evaluated further by peripheral smear. The full diagnostic workup of mixed cell line disorders is varied and beyond the scope of this discussion.4

If isolated anemia is confirmed, a number of additional laboratory tests (included with the CBC) can be used to characterize the patient's red blood cells (RBCs). The mean corpuscular volume is the first branch point in diagnostic reasoning. This step may be followed by the reticulocyte count, red cell distribution width, a Coombs test, or a peripheral smear to further refine the differential. Figure 1 summarizes a stepwise approach to diagnosing the etiology of isolated anemia, recognizing that different disease states may have overlapping features based on laboratory values alone. In practice, several tests will be used simultaneously, and the results should be considered in aggregate to clarify the clinical picture.

Case Continued

In further evaluating this patient's normocytic anemia, the reticulocyte count was found to be elevated at 7.0%. Direct and indirect Coombs testing was negative. A peripheral smear revealed spherocytes and schistocytes. Lactate dehydrogenase was elevated at 4659 U/L; total bilirubin was elevated at 81.1 mg/dL, with a direct bilirubin of 0. A urinalysis of the cola-colored urine revealed large blood, 3 RBCs and large bilirubin. Additional laboratory testing revealed glucose-6-phosphate-dehydrogenase (G6PD) deficiency.

**Question What Are the Current Recommendations for the Evaluation of Hemolytic Anemias in the Pediatric Population?**

**Discussion**

This patient's history and physical examination and laboratory findings (normocytic anemia with an elevated reticulocyte count, elevated lactate dehydrogenase, unconjugated hyperbilirubinemia, schistocytes on smear, hemoglobinuria, and bilirubinuria) were suggestive of a hemolytic disorder.6

Hemolytic processes can be divided broadly into intrinsic (specific RBC defect causing hemolysis) versus extrinsic (destructive processes) etiologies. Figure 2 shows the clinical methodology used to differentiate different types of hemolytic anemias (HAs).

Extrinsic causes of HA can be immune or non-immune-mediated processes; platelet count, presence/absence of coagulopathy, and Coombs testing are essential diagnostics. Microangiopathic HA, which includes disseminated intravascular coagulation and hemolytic-uremic syndrome, comprises most causes of nonimmune extrinsic hemolysis and is critical to identify early in presentation to guide rapid treatment.7 To detect autoimmune HA, Coombs testing is performed across a range of temperatures to detect warm (usually immunoglobulin G) versus cold (predominantly immunoglobulin M) agglutinins. Of note, Coombs-negative autoimmune HA has been described in extremely rare cases. This patient's Coombs testing was negative in both the warm and cold temperature spectrums and, taken in conjunction with the absence of coagulopathy and a normal platelet count, an intrinsic etiology was suspected.

Intrinsic causes of HA can be categorized into hemoglobinopathies, membrane defects, and enzyme deficiencies. A peripheral smear is an essential adjunct to laboratory testing in the evaluation of these disorders.8 Membrane defects underlie conditions such as hereditary spherocytosis and elliptocytosis, which can be readily identified by examining the peripheral smear. However, both spherocytes and elliptocytes may appear in other hemolytic conditions and are therefore not specific for hereditary disorders. Osmotic fragility testing can be used to identify intrinsic RBC membrane instability and guide further testing.9

Hemoglobinopathies such as sickle cell anemia and thalassemias can present with hemolytic crises and thus are also in the differential for this patient. After routine laboratory testing, evaluation for these disorders begins with protein electrophoresis to identify hemoglobin variants and their relative proportions and subsequently progresses to confirmatory testing with DNA or protein analysis.10 A family history can strongly support hemoglobinopathy as an etiology for HA. In this patient, a negative family history and peripheral smear findings led to testing for an enzyme deficiency, with G6PD deficiency being the most common.

G6PD deficiency is one of a number of enzyme deficiencies that can cause HA. It is the most prevalent enzyme deficiency in the world, affecting ~330 million people with a geographic focus in malaria-endemic regions.11 There is also a spectrum of disease depending on the severity of enzyme deficiency, ranging from class I variants (severe deficiency leading to chronic HA) to class II and III (moderate enzyme deficiency with intermittent hemolysis) to class IV and V (no deficiency to increased enzyme activity without clinical manifestations).12 It is possible to directly measure G6PD levels in patients' blood. Of note, during active hemolysis G6PD may be falsely detected as normal or elevated due to the destruction of the severely affected cells and the formation of new RBCs. Levels measured in this patient were significantly decreased despite being untreated at the time of measurement. This finding lends additional support to the diagnosis of true G6PD deficiency.

The primary means to prevent hemolysis in G6PD deficiency is to avoid exposure to drugs, chemicals, or foods, specifically fava beans, known to have oxidant potential. The most common drugs include
FIGURE 1  Sideroblastic anemia can be characterized by elevated iron, decreased TIBC, and Pappenheimer bodies on peripheral smear. Note that iron deficiency may mask classic features of sideroblastic anemia on testing. Reticulocytosis in acute hemolysis/blood loss may cause nonmegaloblastic macrocytosis. Although homocysteine levels will be elevated in both folate and B12 deficiency, increased MMA is fairly specific for B12 deficiency. In healthy patients, reticulocytes comprise ~0.5–1.5% of circulating erythrocytes. This number can be corrected to account for the severity of the patient’s anemia to yield an “expected” value by using the reticulocyte index (observed reticulocyte % × Hgb [0.45]).

Jaundice, hemoglobinuria, bilirubinuria, indirect hyperbilirubinuria, increased lactate dehydrogenase, decreased haptoglobin, schistocytes on peripheral smear; B12, vitamin B-12; Hgb, hemoglobin; FOBT, fecal occult blood test; MCV, mean corpuscular volume; MMA, methylmalonic acid; RDW, red cell distribution width; TIBC, total iron binding capacity; %ile, percentile.
primaquine, dapsone, rasburicase, probenecid, and certain sulfa drugs. The avoidance of methylene blue is of particular relevance to this case and will be discussed below. Fava beans are historically linked to acute hemolysis in G6PD-deficient individuals. The phenomenon that was once known as "favism" was observed when individuals with G6PD deficiency, especially in Mediterranean countries, underwent acute hemolysis after the ingestion of fava beans. It was found that the beans contained oxidants, such as divicine, isouramil, and convicine, which were responsible for promoting hemolysis. The medical management of favism has included the use of splenectomy and, due to its antioxidant effects, vitamin E, with neither of these therapies showing benefit. During acute hemolytic crises, it is imperative to remove the inciting trigger and to monitor the rates of hemolysis and erythropoiesis, with transfusion being indicated if the production of RBCs is unable to keep up with the rate of destruction. In addition, individuals with the class I variant may benefit from folic acid supplementation because they may become deficient given their chronic HA.

Case Continued
The patient's oxygen saturation improved to only 92% with 15 L oxygen via a non-rebreather face mask. In evaluation of the patient's desaturation in the absence of respiratory symptoms and a normal chest radiograph, co-oximetry revealed a PaO2 of 126 mm Hg and an elevated methemoglobin level of 8.3%.

Question What Is the Evaluation of Desaturation in This Clinical Scenario? Is There Any Relationship to the Patient’s Underlying Etiology?

Discussion
Desaturation in the setting of anemia without evidence of lung disease must be further investigated with arterial blood gas testing and co-oximetry. Bedside pulse oximetry is falsely low in the presence of methemoglobinemia, as shown in our patient, because it only measures the absorbance of 2 wavelengths of light (660 and 940 nm) to differentiate oxyhemoglobin from deoxyhemoglobin. By using calibration curves, the ratio of absorption of light at each of these wavelengths is converted into oxygen saturation. A ratio of absorbance of 0.43 correlates to 100% oxygen saturation, whereas an absorbance of 1.0 corresponds to ~85%. Methemoglobin absorbs light at both 660 and 940 nm; thus, in the presence of elevated levels, the ratio is ~1.0 and the oxygen saturation plateaus near 85%.

Arterial blood gas testing with co-oximetry allows for the accurate measurement of methemoglobin and is key to diagnosing methemoglobinemia. A co-oximeter is a simplified spectrophotometer that can measure light absorbance at 4 different wavelengths and can differentiate methemoglobin from carboxyhemoglobin, oxyhemoglobin, and deoxyhemoglobin. A peak absorbance of light at 630 nm is used to characterize methemoglobin. In conjunction with a co-oximeter, an arterial blood gas test can be a more reliable source for oxygen saturation because it reflects the contribution of all 4 types of hemoglobin, leading to a difference in saturation between the pulse oximeter and blood gas, a "saturation gap." The PaO2 would also be normal in patients with methemoglobinemia because it reflects plasma oxygen content and does not correspond to the oxygen-carrying capacity of hemoglobin.
The finding of methemoglobinemia in this case is directly related to the underlying diagnosis of this patient’s anemia. Although rare, the association between methemoglobinemia and G6PD has been reported in several case studies.18 Oxidant injury to hemoglobin can lead to the oxidation of the ferrous iron (Fe2+) in the heme group, leading to iron in the ferric state (Fe3+), resulting in methemoglobin, which is incapable of binding to oxygen.19 Normally, this process is prevented by an enzyme, nicotinamide adenine dinucleotide–dependent cytochrome b5 reductase, which maintains methemoglobin levels at <1% of total hemoglobin. When this system is overwhelmed, however, such as in the case of oxidant stress in G6PD deficiency, the accumulation of methemoglobin cannot be averted. In this patient's case, this process explains his persistently low oxygen desaturation despite supplemental oxygen.

Although elevated levels of methemoglobin are abnormal, treatment is not always indicated. If there are no signs of clinical symptoms or certain comorbidities that could limit one's capacity to withstand lower oxygen-carrying capacity, the removal of the inciting agent can be considered.20 The definitive treatment of methemoglobinemia is methylene blue, which serves as a cofactor for nicotinamide adenine dinucleotide phosphate–dependent methemoglobin reductase to reduce iron from the ferric to ferrous state, thereby transforming methemoglobin to hemoglobin.21 Methylene blue, however, is not without its own complications, because it can lead to systemic and pulmonary hypertension, anaphylaxis, and further hemolysis.20 Treatment is often reserved for cases in which methemoglobin levels exceed 20%, although treatment may be indicated at lower levels if other comorbid conditions exist, such as severe anemia or cardiopulmonary disease.21 In G6PD deficiency, the RBC’s inability to reduce nicotinamide adenine dinucleotide phosphate results in the failure of methylene blue to reduce methemoglobin to hemoglobin, thus limiting its role.19 Furthermore, methylene blue contains oxidative potential in itself, which can lead to further hemolysis and methemoglobinemia. Therefore, as in this patient, methylene blue should be avoided in patients with G6PD deficiency.

Alternative treatments exist, although they are not as quick to act as methylene blue and they are not without their own limitations. Ascorbic acid, better known as vitamin C, is one alternative due to its antioxidant effects.22 Other investigatory therapies include N-acetylcysteine23 and cytochrome P-450 inhibitors in dapsone-induced methemoglobinemia,24 although studies remain limited. Treating the underlying cause behind the production of methemoglobin, such as the oxidative stress in G6PD deficiency, can also be a form of treatment.22 In this case, the gradual improvement in the patient’s anemia led to the resolution of his methemoglobinemia.

Case Continued

The patient was treated with multiple RBC infusions over a few days with improvement in his hemoglobin and hematocrit and resolution of his pallor, fatigue, jaundice, and dark urine. His methemoglobin level also normalized without any other interventions.

On initial questioning regarding dietary history with a Mandarin interpreter, the patient’s mother denied any new foods, and specifically denied ingestion of fava beans, which later the medical team realized she had interpreted as soybeans. Further questioning of the family with a Mandarin informational handout on G6PD with pictures revealed that 1 day before the development of symptoms, the patient had eaten a cup of fava beans and drank a traditional Chinese herbal tea, both of which were illustrated on the handout.

Question How Does Limited English Proficiency Impact Patient Care in Pediatrics?

Discussion

Despite the assistance of Mandarin-speaking interpreters throughout the patient’s admission, it was not until the day of discharge that the medical team was able to elucidate the inciting triggers of the patient’s hemolytic crisis. There are limited data on how English proficiency affects patient safety and objective outcomes in care, because many data collection systems fail to record patients’ race/ethnicity, primary language, and English proficiency. On the basis of existing literature, it is clear that limited English proficiency (LEP) does have a negative impact on patient safety.

Language barriers have been shown to result in insufficient patient history, including omission of important information regarding past medical problems, drug allergies, and the chief complaint.25 Multiple studies have documented that quality of care is compromised when LEP patients require, but do not have access to, interpreter services. In a systematic review by Flores26 in 2005, it was found that patient outcomes were optimized in situations in which LEP patients had access to trained professional interpreters and bilingual providers. Miscommunication may still occur between care providers and families, even with the use of medical interpreters. This patient’s admission revealed an underlying chronic illness that will require long-term management with dietary and medication restrictions. Parental LEP has been independently associated with worse health care access and quality in children with special health care needs.27 Based on the experience with this patient, it is important to ensure that evaluation and anticipatory guidance for LEP patients and families be performed with the consistent use of appropriate interpreters and visual aids.

LEP and low health literacy are distinct, but associated, impediments to health care–related communication. In a study by Sentell and Braun,28 it was shown that both can independently impact patient care and lead to worse health outcomes. Their study also showed that LEP may carry a greater risk than low health literacy. In this case, the patient’s family’s health literacy was never formally tested or even self-reported; however, it is still important to be mindful of this potential impediment in future clinical work.
REFERENCES

**Sometimes, It's Just Black and White: Dark Urine and Pallor in a 2-Year-Old Boy**
Benjamin Wadowski, Denis Chang, Sabina Q. Khan and Tanya Chadha
*Hospital Pediatrics* 2016;6;560
DOI: 10.1542/hpeds.2015-0252 originally published online August 23, 2016;

<table>
<thead>
<tr>
<th>Updated Information &amp; Services</th>
<th>including high resolution figures, can be found at: <a href="http://hosppeds.aappublications.org/content/6/9/560">http://hosppeds.aappublications.org/content/6/9/560</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplementary Material</td>
<td>Supplementary material can be found at: <a href="http://hosppeds.aappublications.org/content/6/9/560#BIBL">http://hosppeds.aappublications.org/content/6/9/560#BIBL</a></td>
</tr>
<tr>
<td>References</td>
<td>This article cites 28 articles, 2 of which you can access for free at: <a href="http://hosppeds.aappublications.org/content/6/9/560#BIBL">http://hosppeds.aappublications.org/content/6/9/560#BIBL</a></td>
</tr>
<tr>
<td>Subspecialty Collections</td>
<td>This article, along with others on similar topics, appears in the following collection(s):</td>
</tr>
<tr>
<td>Blood Disorders</td>
<td><strong>Blood Disorders</strong> <a href="http://www.hosppeds.aappublications.org/cgi/collection/blood_disorders_sub">http://www.hosppeds.aappublications.org/cgi/collection/blood_disorders_sub</a></td>
</tr>
<tr>
<td>Hematology/Oncology</td>
<td><strong>Hematology/Oncology</strong> <a href="http://www.hosppeds.aappublications.org/cgi/collection/hematology:oncology_sub">http://www.hosppeds.aappublications.org/cgi/collection/hematology:oncology_sub</a></td>
</tr>
<tr>
<td>Permissions &amp; Licensing</td>
<td>Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: <a href="http://www.hosppeds.aappublications.org/site/misc/Permissions.xhtml">http://www.hosppeds.aappublications.org/site/misc/Permissions.xhtml</a></td>
</tr>
<tr>
<td>Reprints</td>
<td>Information about ordering reprints can be found online: <a href="http://www.hosppeds.aappublications.org/site/misc/reprints.xhtml">http://www.hosppeds.aappublications.org/site/misc/reprints.xhtml</a></td>
</tr>
</tbody>
</table>
Sometimes, It's Just Black and White: Dark Urine and Pallor in a 2-Year-Old Boy
Benjamin Wadowski, Denis Chang, Sabina Q. Khan and Tanya Chadha
Hospital Pediatrics 2016;6:560
DOI: 10.1542/hpeds.2015-0252 originally published online August 23, 2016;

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hosppeds.aappublications.org/content/6/9/560