A 14-Year-Old Boy with Chronic Cyanosis, Mild Anemia, and Limited Physical Resistance to Stress

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CASE

In April 1997, a then 4-year-old boy suddenly fell ill with fever, cough, fatigue, and poor physical resistance to stress. Marked cyanosis of the lips and the nail beds was noted. During his 3-week hospital stay, mycoplasmosis of the lung and a respiratory syncytial virus infection were diagnosed by the detection of high-titer Mycoplasma agglutinins in serum samples and positive test results for the presence of respiratory syncytial virus in nasopharyngeal secretions. In addition, incipient exogenous allergic alveolitis was suspected because of the presence of budgerigars and cockatiels in the boy’s home and the finding of serum positivity for IgG precipitin. The boy’s condition gradually improved with cefuroxime and clarithromycin treatment and oxygen therapy. α1-Antitrypsin deficiency and cystic fibrosis were excluded. Because the patient’s oxygen saturation failed to improve during sleep and at times dropped to well below 90%, a cardiology examination was performed, and a cardiac defect was ruled out. The reticulocyte count and the curve of the absorption spectrum of oxyhemoglobin and deoxyhemoglobin were within the reference interval. Hemogram results were as follows: leukocytes, 5500/μL (reference interval, 5100–12 900/μL); hemoglobin, 11 g/dL (reference interval, 10.7–13.9 g/dL); thrombocytes, 319 000/μL (reference interval, 200 000–445 000/μL); mean corpuscular volume, 78 fl (reference interval, 74–89 fl); mean corpuscular hemoglobin, 26 pg (reference interval, 24.5–31 pg). At the same time, hemoglobin electrophoresis excluded hemoglobinopathy. One year later, the boy was electively readmitted to the pediatric clinic for assessment of continuous intermittent low oxygen saturation. Exogenous allergic alveolitis was excluded after bronchoalveolar lavage; sarcoidosis was also ruled out. Slight cyanosis of the lips was once again noted, however. Pulse oximetry showed normal oxygen saturation >93% during the day, but oxygen saturation repeatedly dropped to as low as 85% during sleep. Oxygen administration improved saturation immediately to >95%. These fluctuations in oxygen saturation were confirmed by capillary blood gas analysis. When, the mother happened to try out the finger clip of the pulse oximeter on her own finger during the boy’s hospital stay, low oxygen saturation was noted in her as well. Apart from hypertension, she had no preexisting conditions. A physical examination revealed neither cardiac nor bronchopulmonary disease. Hemoglobin electrophoresis revealed no abnormal hemoglobin. The mother reported poor physical resistance to stress with dyspnea and intermittent cyanosis of the lips after minor physical activity, which had been present since childhood. Owing to the still unexplained cause of the low oxygen saturation, the mother was admitted to a pneumology unit to test her once again for a cardiac defect as well as sleep apnea syndrome. None of the tentative diagnoses could be confirmed. She showed a continuously low oxygen saturation of <90%, and pulse oximetry measurements documented >140 desaturations (as low as 71%) during sleep. This finding was eventually interpreted as a faulty pulse oximetry measurement, however, due to poor acral circulation. Psychiatric treatment was recommended despite the fact that a capillary blood gas analysis also had revealed low oxygen saturation. Because physical resistance to stress continued to be very poor in both the mother and son, the family’s general practitioner contacted us after conducting intensive research, because we had previously described a new hemoglobin variant, hemoglobin Bonn, which produces falsely low oxygen saturation measurements (1).

QUESTIONS TO CONSIDER

1. What possible causes for peripheral cyanosis exist once pulmonary and cardiac reasons have been excluded?
2. What examinations must be carried out if a hemoglobin anomaly is suspected?
3. What limitations exist for identifying hemoglobin variants?
DISCUSSION

PATIENT FOLLOW-UP

A falsely decreased oxygen saturation by pulse oximetry could be excluded by spectrophotometric measurement of oxyhemoglobin and deoxyhemoglobin (2). For both patients, a hemoglobin HPLC analysis with a Variant II instrument (Bio-Rad Laboratories) revealed clear peaks of 32.3% and 32.8% directly behind A0 (retention time, 2.58 min; Fig. 1). For both mother and son, HPLC analysis noted increased hemoglobin A1c (Hb A1c) values (7.4% and 7.5%, respectively; nondiabetic reference interval, <5.7%; diabetes cutoff, ≥6.5%), despite normal glucose results. The Hb A1c percentages obtained by immunoassay (Dimension Vista; Siemens) were 5.9% for the boy and 5.4% for the mother (nondiabetic reference interval, <5.7%; diabetes cutoff, ≥6.5%). Hemoglobin gel electrophoresis (Hydrasys; Sebia) was performed at pH 6.0 and pH 8.5. No abnormalities were found. Hemoglobin capillary electrophoresis (Capillaries; Sebia) also revealed no abnormalities. A sample of arterial blood analyzed by co-oximetry for oxygen saturation showed a normal oxygen pressure of 94 mmHg (reference interval, 70–100 mmHg) and a decreased arterial oxygen saturation value of 84% (reference interval, >96%). Methemoglobin and carboxyhemoglobin values were normal. The partial pressure of O2, at which hemoglobin is half-saturated (P50), in whole blood was measured with a blood gas analyzer and found to be increased [39 mmHg: normal, 26 mmHg (1)].

Because the HPLC results suggested an inherited hemoglobin variant, blood samples were collected from family members into tubes containing EDTA. Genomic DNA was isolated from the blood samples with the QIAamp DNA Blood Kit (Qiagen). Amplification of HBB (hemoglobin, beta) exons was carried out by the PCR (oligonucleotide sequences available on request). For mutational analysis, PCR-amplified DNA products were subjected to direct automated sequencing (373A DNA Sequencer; Applied Biosystems). Initially, both strands of the patients’ amplicons were sequenced, and segregation of the mutation in family members was investigated by sequencing the respective PCR products for exon 2. Sequencing of the HBB gene in mother and son revealed a single c.255C>T transition (GenBank accession no. NM_000518.4) in exon 2 of the HBB gene, yielding a p.Leu68Phe substitution. This mutation has not been described to date, and we termed it “hemoglobin Venusberg.” Isoelectric focusing was not carried out. No abnormalities with hemoglobin Venusberg can be assumed, however, because none have been noted for 2 other hemoglobins with mutations at the same position [hemoglobin Brisbane (p.Leu68His) (3) and hemoglobin Mizuho (p.Leu68Pro) (4)]. Mutations in this area of the β-chain are located within the heme-binding pocket, which is important for oxygen binding (5).

Hemoglobin anomalies with low oxygen affinity are rare; however, they can often lead to low oxygen saturation, peripheral cyanosis (6), anemia, and hemolysis (7). In the hemoglobin molecule, α and β dimers are structurally stabilized in the deoxy form (T structure) in solid form via salt bridges. With increasing oxygenation, the salt bridges between the α and β subunits are broken down to produce a predominant oxy form (R structure). A change in amino acid residues can cause an imbalance between the R and the T structures. This impaired cooperation between the subunits is postulated to be the most common cause of modified oxygen affinity of the hemoglobin molecule. In this case, the deoxy form (T structure) in particular is stabilized (8). The exact mechanism, however, has not been fully defined, yet an amino acid change at position 68 of the β-globin chain also shows a change in the oxygen affinity of hemoglobin Bris-

Fig. 1. Hemoglobin chromatogram of hemoglobin Venusberg showing a clear peak at 2.58 min and hemoglobin electrophoresis (E) results showing no apparent electrophoretic abnormalities.

The anode (+) is indicated at the top of each of the electrophoresis gel images. Co. ASFC, control hemoglobins A, S, F, and C.

3 Nonstandard abbreviations: Hb A1c, hemoglobin A1c; P50, partial pressure of O2, at which hemoglobin is half-saturated.
Cyanosis and Low Oxygen Saturation

The general causes of cyanosis are pulmonary, cardiac, and vascular diseases. Other possible causes include formation of methemoglobin due to medication (nitrate, dapsone, and so forth), enzymatic deficiency (deficiency in cytochrome b₅ reductase or cytochrome b₅), Hb M anomalies, unstable hemoglobin variants, and easily oxidizable variants. Other rare causes include sulfhemoglobin (hydrogen sulfite poisoning, sumatriptan) and, as in this case, hemoglobin variants with reduced oxygen affinity (6). In many cases of rare hemoglobin anomalies, the final diagnosis was reached only after an extended period of physical examination and laboratory assessment. Low oxygen saturation is primarily thought to have cardiologic or pulmonary causes. In adults, sleep apnea syndrome is often suspected (1). Diagnosis can be difficult, however. Occasionally, laboratory errors are assumed, or psychosomatic causes are suspected.

Falsely high Hb A₁c values often occur in persons with hemoglobin variants, often leading in turn to a diagnosis cascade to rule out diabetes mellitus. Falsely low Hb A₁c values in HPLC analyses have also been described for a hemoglobin variant with a low oxygen affinity (10). On the other hand, the low oxygen saturation in the mother or son in this case might be misinterpreted during an emergency, intraoperatively, or in intensive care. A high P₅₀ indicates a rightward shift in the oxygen dissociation curve, i.e., a low oxygen affinity. In a case of low oxygen saturation of unknown origin, the possibility of an anomalous hemoglobin variant with a low oxygen affinity must be taken into consideration. In such a case, the P₅₀ is the most diagnostically conclusive. It is also a diagnostic criterion for hemoglobin Venusberg. The name “hemoglobin Venusberg” was chosen to mark the place of the first discovery of this hemoglobin, the location of University Clinics Bonn. In the present case, hemoglobin electrophoresis was carried out twice in both patients, which led to the erroneous exclusion of a hemoglobinopathy. Not all hemoglobin variants produce changes in charge that are detectable by hemoglobin electrophoresis. If a hemoglobinopathy is suspected, several detection methods should be applied, if possible. Hemoglobin Venusberg is clearly detectable as a peak in hemoglobin chromatography (Fig. 1).

Points to Remember

- Peripheral cyanosis may be caused by pulmonary, cardiac and vascular diseases, enzyme deficiency, methemoglobinemia, sulfhemoglobinemia, and hemoglobin anomalies.
- Some hemoglobin anomalies are not detected by hemoglobin electrophoresis. Therefore, if a hemoglobin anomaly is suspected, genetic analysis should be carried out in addition to hemoglobin chromatography.
- Symptoms of hemoglobin Venusberg include intermittent drops in oxygen saturation, especially during sleep, intermittent slight cyanosis of the lips and the onychodermal, limited physical resistance to stress, falsely high Hb A₁c values in HPLC analyses, normal hemoglobin electrophoresis and capillary electrophoresis findings, but a clear peak of 32% in hemoglobin chromatography. The different modalities of hemoglobin analysis, electrophoresis, and HPLC have their own particular advantages and disadvantages. Sequencing of the HBB gene revealed a single c.255C>T transition in exon 2 of the HBB gene, yielding a p.Leu68Phe substitution.

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Commentary

Mary Frances McMullin*

This fascinating and carefully worked out case study describes a boy originally presenting with cyanosis at the age of 4 years. He and his mother were found to have a newly described low-affinity hemoglobin, termed “hemoglobin Venusberg.” Of note are the extensive investigations carried out on both individuals before the diagnosis was obtained, the fact that this variant was detected not by hemoglobin electrophoresis but by the presence of an abnormality in hemoglobin chromatography, and the repeated low oxygen saturations revealed by pulse oximetry. Both individuals were reported as having “limited physical resistance to stress,” which has not been reported before in the literature with low-affinity hemoglobin variants, and it is not clear why such a variant should lead to limited exercise tolerance.

Oxygen is carried in the blood bound to hemoglobin, and each type of hemoglobin has a certain capacity for holding oxygen. In the lungs, the partial pressure of oxygen is high, and oxygen binds to hemoglobin, whereas in the tissues the partial pressure is lower, and the oxygen is released as required. This relationship between the partial pressure of oxygen and the oxygen saturation of hemoglobin is described in the oxygen-dissociation curve. With normal hemoglobins under normal conditions, hemoglobin is 50% saturated at a partial pressure of oxygen of 26.6 mmHg (the P50). The curve is shifted to the right to give a higher P50. That means that a larger partial pressure is required to maintain 50% saturation, and there is decreased oxygen affinity. A hemoglobin such as hemoglobin Venusberg has an alteration that leads to lower oxygen affinity and thus a right-shifted oxygen dissociation curve.

It is important to consider these hemoglobin variants in such a case to explain the cyanosis and to try to avoid unnecessary investigation. No treatment is required.

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