globin reductase deficiency (since methemoglobin A is produced in these instances).

This case confirms that definitive identification of a hemoglobin variant is not always possible using HPLC and electrophoresis, even when both techniques are used in parallel. Indeed, with the use of these techniques alone, Hb M-Boston could easily be mistaken for Hb Q-India. To our knowledge, this is the first description of Hb M-Boston in a patient of Indian ethnicity. Given the lack of evidence demonstrating a causative link between Hb Q-India and methemoglobinemia, it is tempting to speculate that Hb M-Boston may actually be more prevalent in Indian populations, yet is being misidentified as Hb Q-India.

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Commentary

James D. Hoyer*

This case highlights 3 very valuable points. These are the importance of correlating laboratory findings with the clinical presentation, the value of multiple methods for the identification of hemoglobin variants, and the appropriate use of molecular testing.

With the patient's clinical presentation, family history, abnormal pulse oximetry readings, and the presence of a hemoglobin variant on electrophoresis, the authors are commended for strongly suspecting an M hemoglobin. However, they were led astray by the HPLC results, making an identification of the variant as Hb Q-India even though the acid electrophoresis migration was inconsistent and Hb Q-India is not associated with clinical symptoms.

Fortunately, the authors sent the sample for molecular confirmation in which the hemoglobin variant was identified as Hb M-Boston, a result fully consistent with the clinical presentation. The authors indicate that the HPLC retention time of HB M-Boston is not known; however, a very large series of the retention times of hemoglobin variants was published in 2012, which included 6 cases of Hb M-Boston, with molecular confirmation *(1)*. In that study the retention time of Hb M-Boston (mean retention time, 4.80

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min; range 4.74–4.85 min) was consistent with the retention time in this case. The slight difference in retention time may be because the study described in *(1)* compiled retention times on the Bio-Rad Variant Classic, and not the Bio-Rad Variant II, as reported here. In addition, Hb M-Boston does migrate close to the Hb S position on acid electrophoresis.

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Commentary

Neil S. Harris,* Stacy G. Beal, and William E. Winter

In the atmosphere at 20 °C, oxygen has a concentration of approximately 8.8 mmol/L, which matches the concentration of hemoglobin monomers in the blood. Therefore, when fully saturated, hemoglobin allows blood to achieve "atmospheric" oxygen concentrations.

Hemoglobin is remarkable for its exquisitely fine-tuned binding site that allows binding of oxygen without immediately oxidizing the ferrous iron (Fe^{2+}) to the ferric form (Fe^{3+}), while at the same time diminishing the very high binding affinity of carbon monoxide (CO), which is continuously generated by heme degradation. The oxygen binding site contains the heme porphyrin ring, which is bound to Fe^{2+}, forming a pentacoordinate complex: 4 covalent bonds from the porphyrin and 1 from the "proximal" (F8) histidine. Binding of oxygen is to the sixth coordination position of heme; this binding places the oxygen at an angle relative to the plane of the heme and allows the oxygen to hydrogen bond to the so-called "distal" (E7) histidine.

The hemoglobin M variants arise mainly as a result of the substitution of the proximal or distal histidines by tyrosine. These include Hb M-Boston and Hb M-Saskatoon (α and β chain distal histidine substitutions, respectively) and in Hb M-Iwate and Hb M-Hyde Park (α and β chain proximal histidine substitutions). Furthermore, there is an important role for a valine residue on the β chain, demonstrated in Hb M-Milwaukee, in which valine-E11 is replaced by glutamic acid. Hemoglobin M is not only resistant to the normal reduction mechanisms; in hemoglobins Boston and Iwate (both α substitutions), the oxygen affinity of the normal β subunit is very low.

Hemoglobin M variants present a challenge to the clinical chemist because they likely will be misread by oximeters, as described in this case. Our interpretations are only as good as our understanding of the biology of disease.

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