A Patient with a Previous Diagnosis of Hemoglobin S/C Disease with an Unusually Severe Disease Course

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CASE

A 17-year-old African American male presented to the hematology clinic for treatment of sickle cell disease (SCD).1 He had received the diagnosis of hemoglobin (Hb) S/C disease at an outside hospital at the age of 6 years; the diagnosis was confirmed in house at 11 years of age. His disease course had been severe, with frequent pain crises of increasing intensity and 2 episodes of acute chest syndrome requiring hospitalization and multiple blood transfusions.

The patient’s physical examination was unremarkable: blood pressure, 120/64 mmHg; pulse, 83 beats/min; temperature, 36.9 °C. Laboratory results were as follows: white blood cell count, 11.8 × 10^9/L [reference interval (RI), 3.9–10.3 × 10^9/L]; Hb, 6.39 mmol/L (RI, 8.68–10.8 mmol/L); packed cell volume, 0.28 (RI, 0.42–0.50); red blood cell count, 3.59 × 10^12/L (RI, 4.5–6.0 × 10^12/L); platelet count, 417 × 10^9/L (RI, 135–370 × 10^9/L); mean corpuscular volume, 78 fl (RI, 83–102 fl); mean corpuscular Hb, 28.7 pg (RI, 27–31 pg); mean corpuscular Hb count, 368 g/L (RI, 320–340 g/L); red cell distribution width, 17.3% (RI, 11.5%–14.5%); and absolute reticulocyte count, 0.115 (RI, 0.02–0.10). A peripheral blood smear showed scattered target and sickle cells, rare nucleated red cells, and mild anisopoikilocytosis. Results for the qualitative sickle cell solubility test were positive. Considering the severe disease course, Hb analysis by HPLC and isoelectric focusing (IEF) was ordered (Fig. 1).

DISCUSSION

The family of SCDs, which is characterized by Hb S (Glu6Val substitution in the β-globin protein), is prevalent among African Americans. This substitution decreases the solubility of deoxygenated Hb and leads to the formation of rigid polymers that induce red cell sickling. Sickle cells undergo hemolysis and cause microvascular occlusions that lead to ischemic injury. Inheritance of one Hb S mutation, sickle cell trait, is clinically silent. Inheritance of 2 β alleles, sickle cell anemia (S/S disease), is debilitating, with severe pain crises, increased susceptibility to infection, cerebrovascular events, and chronic organ damage. Patients with S/S disease have severe anemia (Hb, 3.7–6.2 mmol/L) with sickle and target cells on peripheral blood smears. The SCD family also includes hemoglobinopathies of varying severities in which Hb S is coinherited with Hb C, Hb D, Hb E, or Hb O (1). SCD is treated with hydroxyurea, which effectively reduces pain crises and other clinical manifestations. The US Food and Drug Administration has approved hydroxyurea for use in adults, and its efficacy has been demonstrated in adolescents as well (1).

SCD is diagnosed by the measurement of substantial amounts of Hb S by at least 2 separation methods, including HPLC and an electrophoretic method, such as IEF, cellulose acetate, or citrate agar. Because many Hbs coelute or comigrate on HPLC or IEF, respectively, it is crucial that multiple methods be used to confirm suspected hemoglobinopathies. The presence of hemolytic anemia with sickle and target cells on a blood smear and a positive result in a sickle cell solubility test in an African American is consistent with SCD. The present patient’s severe clinical course early in life and his Hb profiles suggested that his Hb S/C diagnosis was incorrect.

PATIENT FOLLOW-UP

Analysis by IEF showed the presence of Hb S and another Hb migrating near the position for Hb C. An HPLC analysis revealed the following: Hb A, <1% (RI, >94%); Hb A2, 3.5% (RI, 2.0%–3.8%); Hb F, 5.9% (RI, <2.0%); Hb S, 42.1% (RI, none); and Hb Other, 47.5% (RI, none). The other Hb eluted in the C window at 4.93 min. The distinctive Hb profiles in the IEF and HPLC analyses suggested 3 potential compound heterozygous hemoglobinopathies; Hb S/C, Hb S/CHarlem, or Hb S/OArab. Further discussion with the patient revealed a family history that included a brother with a recent diagnosis of Hb S/OArab which had originally been misdiagnosed as Hb S/C disease.
In both cases, the methods used for the initial diagnoses are unknown. Hb C and Hb OArab comigrate on IEF and cellulose acetate electrophoresis (Table 1), and past HPLC methodologies were not capable of separating the 2 Hb variants. Citrate agar electrophoresis was the only method capable of differentiating Hb C and Hb OArab. The misdiagnoses of Hb S/C disease in both brothers could have been avoided had citrate agar electrophoresis been used for diagnosis and confirmation in each case.

**DIAGNOSIS**

The diagnosis was Hb S/OArab disease.

**Hb S/C Disease**

Like the Hb S trait, Hb C (βGlu6Lys) heterozygotes have no clinical symptoms. Homozygotes have mild anemia without sickling. Hb S/C disease (coinheritance of Hb S and Hb C) is clinically significant. The red cells of S/C disease are severely dehydrated, causing mild microcytosis and crystal formation. Hb S is concentrated and polymerized in dehydrated red cells, and this process leads to complications. Generally, the clinical course of S/C disease is less severe than S/S disease. Painful episodes begin later in life, occur at less than half the frequency of S/S disease, and appreciable pathology typically manifests after 20 years of age.

Patients with S/C disease have mild anemia and distinctive peripheral blood smears with target cells, "boat-shaped" cells, and S/C poikilocytes. Results for sickle cell solubility tests are positive. S/C disease is diagnosed by detection of Hb S and Hb C in a 1:1 ratio. Hb OArab and Hb CHarlem appear similar to Hb C by IEF and HPLC (Table 1); therefore, citrate agar electrophoresis should be used to distinguish these variants. There is no specific treatment for S/C patients.

**Hb S/CHarlem**

Hb CHarlem (βGlu6Val, Asp73Asn) is a rare double mutation of the β-globin gene that produces sickling disorders in homozygous and compound heterozygous (Hb S/CHarlem) individuals. Both disorders are clinically severe.

Hb S/CHarlem patients have moderate hemolytic anemia, and blood smears show target and sickle cells. S/CHarlem disease is diagnosed by the detection of equal amounts of Hb S and Hb CHarlem via multiple separation techniques (Table 1). The severity of Hb S/CHarlem disease may prompt physicians to treat patients with hydroxyurea.

**Hb S/OArab**

Hb OArab (βGlu121Lys) has a prevalence of 1 in 30,000. Hb OArab heterozygotes are asymptomatic, and homozygous individuals have hemolytic anemia with...
febrile illnesses. Coinheritance of Hb S and Hb OArab produces clinically severe disease, with hemolytic anemia, jaundice, vaso-occlusive complications (pain crises and stroke), pneumonia, acute chest syndrome, and sepsis (4, 6). Sickle and target cells, polychromasia, and sometimes Howell–Jolly bodies are detected on peripheral blood smears. Results of sickle cell solubility tests are positive. HPLC, IEF, and citrate agar electrophoresis (Table 1) all detect Hb S and Hb OArab in equal amounts (4). Because clinicians expect a more severe disease course in S/OArab disease, treatment may be more readily escalated to the use of hydroxyurea compared with S/C disease, which typically features fewer and more mild complications, particularly before 20 years of age.

PATHOPHYSIOLOGY OF Hb S/OArab DISEASE
Hb S/OArab and Hb S/S diseases are clinically similar. Hb OArab copolymerizes with Hb S in red cells. Like Hb S/S, Hb S/OArab has reduced oxygen affinity and a lower gelling point for concentrated deoxygenated Hbs (7, 8). When deoxygenated, Hb S/OArab induces irreversible sickling of red cells (6, 7), which are hemo-
lyzed or cleared through the reticuloendothelial system. Sickled cells block the narrow capillaries, causing membrane damage and vaso-occlusive events (4).

RESOLUTION OF THE CASE
Distinguishing Hb S/C, Hb S/OArab, and Hb S/CHarlem diseases in the laboratory is challenging. Dehydrated red cells of S/C disease are typically microcytic (2), whereas patients with S/OArab disease are often normocytic. As with our patient, microcytosis is seen in some patients with S/OArab disease (6).

IEF revealed Hb S in equal proportion with another Hb that comigrated near Hb C (Fig. 1A). Hb A2, Hb E, Hb CHarlem, and Hb OArab all migrate in the same area. Hb A2 rarely constitutes >10% of the total Hbs. HPLC can differentiate Hb OArab from Hb C, Hb CHarlem, and Hb E. The patient’s HPLC profile (Fig. 1B) shows 2 main Hbs eluting in the S and C windows. The Hb in the C window eluted at 4.93 min, compared with 5.19 min for the Hb C standard. The retention times of Hb variants on the Bio-Rad Laboratories Variant II system are 4.91 min for Hb OArab vs 5.18 min for Hb C (9). Hb E and Hb CHarlem elute at 3.69 min (9) and 4.89 min (personal communication), respectively (Table 1). A minor peak of unknown significance appears after Hb A2 on chromatographs of Hb OArab patients (10) and in the profile of our patient, but not in Hb CHarlem patients. Distinguishing Hb OArab from Hb CHarlem requires citrate agar electrophoresis, in which Hb OArab migrates between the A and S calibrators, whereas Hb CHarlem migrates with Hb S (Table 1). In 2002, the patient was misdiagnosed with S/C disease. The patient’s Hb profile was determined by IEF and HPLC with the Bio-Rad Variant I instrument, which could not separate Hb C and Hb OArab. Citrate agar electrophoresis should have been used to confirm the diagnosis. Differentiation between S/C and S/OArab diseases is now possible with newer HPLC systems (10). Patients whose diseases were diagnosed before implementation of this technology may have received the wrong diagnosis if citrate agar electrophoresis was not used. Sequencing of the present patient’s β-globin gene confirmed a heterozygous mutation at nucleotide 414 (G→A) associated with Hb OArab.

After the rediagnosis, the patient was started on hydroxyurea (1000 mg/day) in accordance with recent NIH consensus documents recommending hydroxyurea treatment in several sickle cell syndromes, including S/C and S/OArab, to reduce such severe disease manifestations as pain crises and acute chest syndrome (1). At follow-up, the patient reported improved health. His anemia had improved slightly (Hb, 7.1 mmol/L; packed cell volume, 0.31; mean corpuscular volume, 84 fL).

We recommend that patients with abnormally severe S/C disease before the age of 20 years be evaluated for Hb S/OArab. These 2 diseases can be differentiated in the laboratory when both new-generation HPLC and either IEF or citrate electrophoresis are used. Sequencing of the β-globin gene confirmed the diagnosis. In this case, an accurate diagnosis, although not essential, prompted a change in treatment strategy. An earlier diagnosis of S/OArab disease may have encouraged cli-
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POINTER TO REMEMBER

- Symptoms of Hb S/OArab disease such as pain crises, infection, and hemolytic anemia are severe and begin early in life. Complications of Hb S/C are significantly fewer in number and have a later onset.
- Patients with a diagnosis of Hb S/C disease and an unusually severe disease course early in life should be evaluated for Hb S/OArab or Hb S/C Harlem disease.
- Hb OArab can be differentiated from other hemoglobinopathies with new-generation HPLC profiling in combination with IEF or citrate agar electrophoresis.
- β-Globin sequencing can confirm a suspected diagnosis of Hb S/OArab disease.
- Whereas S/C disease is usually mild and normally does not require invasive treatments before 20 years of age, S/OArab disease is a severe sickling disorder, and patients often receive treatment similar to those with S/S disease.

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Commentary

Carlo Brugnara

Much progress remains to be made in our understanding of the factors underlying the clinical manifestations of sickle cell disease. The severity of the disease has long been known to be highly variable, with some patients experiencing extremely severe disease and major organ-specific complications early in their lifetimes, and other patients with clinically silent disease who receive their diagnoses much later in life and have an almost normal life expectancy.

Epidemiologic and clinical data have suggested that increased concentrations of hemoglobin F (Hb F) are associated with a decreased severity of the disease. White blood cell counts are also an important predictor of mortality and morbidity—both in sickle cell disease and in the general population—with a shortened life expectancy being associated with higher counts.

The case presented in this issue of Clinical Chemistry demonstrates the remarkable effect of double heterozygosity for Hb S and Hb OArab on the clinical severity of the disease, compared with the more frequently observed Hb S/C double heterozygote. The worse effect of Hb OArab has been attributed to enhanced polymerization of Hb S and to the accompanying dehydration of erythrocytes imposed by the presence of the positively charged Hb OArab variant. This
case also highlights the important role that the laboratory plays in the diagnosis and treatment of this disease. It is only thanks to the astute clinical and laboratory insights of the team taking care of this patient that the initial diagnosis of Hb S/C disease was questioned and the appropriate studies were conducted to reach the final, proper diagnosis. Although substantial progress has recently been made in modeling the risk for stroke (1) or death (2) in individual patients with sickle cell disease, this case highlights the crucial role of clinical reasoning and proper laboratory investigation when dealing with unexpected complications in particular patients.

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References

Commentary
James Hoyer

This case illustrates 2 very important points about laboratory testing for hemoglobin (Hb) disorders. The first is that most methods used for Hb identification are not sufficient as single, stand-alone methods. The only exceptions to this rule are DNA sequencing and mass spectrometry, but these methods are not used by many clinical laboratories because of the expense involved. It has long been a requirement in the College of American Pathologists checklist for hematology that Hb variants, particularly in the S and A2 positions, be confirmed by a second method. As illustrated by this case, performance of acid electrophoresis would clearly have shown that the variant in the Hb A2 position was not Hb C (Fig. 1). Acid electrophoresis is easily able to distinguish between the 3 major variants that migrate in the A2 position: Hb C, Hb E, and Hb OArab. Other combinations of methods, however, can accomplish the same result.

Second, this case emphasizes that Hb analysis should not be interpreted in isolation but must be cor-

Fig. 1. Comparison of Hb S/C and Hb S/OArab after acid electrophoresis.
(A), Hb S/C, with bands in the C and S positions. (B), Hb S/OArab with the second band between the S and A positions.

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related with the patient’s clinical situation and other laboratory findings. The severe clinical course seen in this patient is highly unusual for Hb S/C disease. Furthermore, as was outlined, Hb S/C disease has very characteristic morphologic findings in peripheral blood smears. True sickle cells are rare in Hb S/C disease; their presence in this case was another reason to question the diagnosis. In cases such as this one, confirmation of a reported diagnosis is warranted, particularly if previous records are not available or if there are clinical inconsistencies.

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