CASE DESCRIPTION

A 12-year-old White female with a history of sickle cell trait (HbAS), splenectomy, frequent pain crises, infections, and acute chest syndrome (ACS) presented to the emergency department (ED) with complaints of severe back and leg pain. She was administered morphine for her pain and was discharged upon improvement. Within 24 h, she returned to the ED with labored breathing, chest tightness, and pain. At this presentation, her physical examination was significant for tachypnea and tachycardia with sinus rhythm. A chest X-ray revealed infiltrates in her lower pulmonary lobes and she was diagnosed with severe ACS. She was administered morphine, fluids, ceftriaxone, ondansetron, and oxygen (2 L/min). While in the ED, she acutely decompensated, with increased oxygen requirement (2 to 4 L/min), became febrile, and experienced intensifying pain. A complete blood count (CBC) was performed and revealed leukocytosis, decreased red cell count, increased reticulocyte count, and normocytic, normochromic anemia (Table 1). Her peripheral blood smear also revealed moderate anisopoikilocytosis, scattered macrocytes, codocytes, sickle cells, and rare nucleated red blood cells (Fig. 1, A). Her total bilirubin was increased (1.2 mg/dL; reference interval 0.1 to 0.7 mg/dL); however, no other markers of hemolysis were measured. Based on her clinical history, presentation, and abnormal CBC findings, hemoglobin (Hb) analysis was ordered to confirm her reported carrier status of HbAS. The Hb analysis was performed on a HPLC BioRad Variant β-thal Short Program and showed 54.3% hemoglobin A (HbA), 38% hemoglobin S (HbS), 3.8% hemoglobin A2 (HbA2), and 3.9% hemoglobin F (HbF) (Fig. 1, B). These results prompted the care team to reach out to the clinical laboratory to discuss the case in further detail.

QUESTIONS TO CONSIDER

1. What are the common symptoms of patients with sickle disease and sickle cell trait?
2. What methods are routinely used for detecting hemoglobinopathies in the clinical laboratory?
3. Does the clinical picture support her diagnosis of sickle cell trait?

CASE DISCUSSION

Sickling disorders, or sickle cell disease (SD), comprise a group of autosomal co-dominant hemoglobinopathies characterized by mutations in the gene encoding the Hb β-subunit (1). They occur in patients with two mutant β-globin alleles, with at least one sickle Hb (HbS) allele. The HbS allele is a variant of normal adult HbA, in which a point mutation in codon 6 of the β-globin gene (GAG to GTG) results in the substitution of glutamic acid by valine. Common SD genotypes include HbSS, HbSC, and HbS-β-thalassaemia (1). These combined mutations favor polymerization of the Hb molecule causing red cells to assume a sickle shape that can lead to in vivo hemolysis and vaso-occlusion (1, 2). Acute symptoms of SD include pain crises, ACS, and stroke, with patients developing multi-organ damage over time that leads to substantial disability and shortened life expectancy (1, 2).

In contrast to SD, HbAS is generally benign and asymptomatic (3). Sickling is uncommon in HbAS but can be precipitated by hypoxia, dehydration, increased sympathetic outflow, hyperthermia/hyperthermia, increased 2,3-diphosphoglycerate, and inflammatory cell release (3). In rare cases, these patients may present with hematuria, rhabdomyolysis, chronic kidney disease, renal medullary carcinoma, and pulmonary embolism (3).

LABORATORY TESTING FOR HEMOGLOBINOPATHIES

As of 2006, hemoglobinopathy screening for HbS was included in the United States Recommended Uniform Screening Panel for state and territorial...
newborn screening (NBS) (2). NBS for hemoglobinopathies is performed on dried blood spots using protein-based separation techniques, including gel or liquid electrophoresis, isoelectric focusing (IEF), and HPLC (4). Abnormal NBS hemoglobinopathy screens are confirmed in venous blood specimens by capillary zone electrophoresis (CZE), acid and/or alkaline electrophoresis or IEF in combination with CBC and peripheral blood smear reviews (4). Sequencing of the α- and β-globin gene clusters can differentiate variants with similar migration patterns in protein-based methods (4).

Protein-based separation for Hb analysis relies on amino acid substitutions that result in changes in polarity and overall molecular net charge. An under-recognized and important limitation of protein-based Hb analysis is that electrophoretically silent Hb variants can be misidentified or remain undetected (5, 6). Electrophoretically silent Hb variants result from amino acid substitutions that do not alter the polarity or net charge of the molecule. For example, a benign β-globin chain variant, Hb Québec-Chori results from the substitution of isoleucine by threonine at position 87. When present in combination with HbS, Hb Québec-Chori (“Chori” is an acronym for the Children’s Hospital Oakland Research Institute) promotes Hb polymerization and red cell sickling due to increased donor-acceptor interaction at the site of the substitution (5, 6). Individuals with the HbS/Hb Québec-Chori genotype commonly show HbAS patterning by protein-based separation techniques, as Hb Québec-Chori co-migrates with HbA (5, 6). Published case studies report that HbS/Hb Québec-Chori patients can experience severe ACS and vaso-occlusive pain crises that are responsive to hydroxyurea (5, 6). The College of American Pathologists guidelines stipulate that samples positive for HbS by any method, must undergo confirmatory testing by a second method.

**RACE AND HEMOGLOBINOPATHIES**

Notably, the patient’s White race is included in the case presentation, which highlights an important question: should a patient’s race influence the index of suspicion for genetic disorders that cluster by ancestry, such as SD? Genetic ancestry is often inferred using surrogates such as skin color, race, and ethnicity. These surrogates may be included in clinical case presentations and considered when formulating differential diagnoses for hereditary diseases that show differences in prevalence between different ancestries (7). The practice of using race in clinical case presentations is rooted in racial segregation of healthcare during the Jim Crow Era (7). More importantly, as race and ethnicity are socio-political constructs rather than distinct biological categories, they should not be conflated with genetic ancestry, which is a biological, measurable classifier (7).

By analyzing ancestry-informed markers or clustering of ancestral markers, individuals’ genetic ancestry can be assigned (8). These analyses have revealed that humans cluster geographically based on historic continents and migration patterns. The population of the US as it exists today developed through historic and ongoing admixture between groups with different geographic ancestry, which has led to increasing genetic heterogeneity in the US. While self-reported race often correlates closely with major genetic ancestry, one study found a 3.6-fold increase in carrier rate for β-globin hemoglobinopathies in patients who did not self-report as Black/African American or African but had a substantial proportion of genetic African ancestry (9). Simply put, the presence or absence of a genetic sequence cannot be inferred based on a patient’s reported race. Based on this premise, in 2021 the American College of Medical Genetics and Genomics recommended that carrier screening paradigms should be ethnic and population neutral (10).
Clinical Case Study

Fig. 1. (A), Peripheral blood smear; (B), cation-exchange HPLC chromatogram; (C), alkaline electrophoresis gel; (D), isoelectric focusing gel; and (E), acid electrophoresis gel.
CASE CONCLUSION

At birth, this patient’s NBS was abnormal for HbAS, which was subsequently confirmed by HPLC. On 2 separate hospital presentations, additional HPLC and IEF Hb evaluations were performed and interpreted as consistent with HbAS; however, the patient’s clinical picture with frequent pain crises, infections, and ACS were atypical for someone with HbAS. It is unclear whether the patient’s non-African American race was considered non-contributory during her previous admissions and repeated laboratory investigations. It is possible, however, that considering the patient’s race in evaluating the likelihood of SD delayed further analysis and accurate diagnosis.

On the current admission, additional analysis by acid and base electrophoresis was also consistent with HbAS (Fig. 1). The consult revealed that her father was a known sickle cell carrier of Native American ancestry, whereas her mother had a history of “abnormal Hb,” unknown type, which lead the clinical team to suspect that a silent sickling Hb variant was present. Emergent red blood cell exchange (RBCEx) was proposed and performed in the setting of ACS and led to clinical improvement. Further analysis by genetic sequencing was recommended and the results confirmed that the patient was heterozygous for HbS/Hb Québec-Chori.

This case illustrates the need for further investigation when the clinical presentation is incongruent with the results of routine laboratory testing, rather than dismissing the case based on a racial profile. This is especially imperative for silent sickling Hb heterozygous patients who can present with imminent stroke or ACS, entities that require emergent RBCEx therapy.

POINTER TO REMEMBER

- Race and ethnicity are socio-political constructs that should not be conflated with genetic ancestry.
- Clinical presentations suggestive of SD in patients diagnosed with sickle cell trait need to be carefully evaluated to exclude the possibility of electrophoretically silent Hb sickling variants that may be missed by routine protein separation-based methods.
- Genetic sequencing is the gold standard for confirmation of Hb variants that cannot be resolved by protein-based separation techniques.