Compound Heterozygous Hemoglobinopathy Complicated by Inaccurate Capillary Electrophoresis Zoning

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CASE DESCRIPTION

A 23-year-old male underwent hemoglobinopathy evaluation due to a positive sickle cell solubility screen performed as part of a sports physical exam. Relevant complete blood count indices included: borderline increased erythrocyte count (red blood cell count) of 5.73 × 106/µL [reference interval (RI) 4.4–5.7 × 106 µL], mildly decreased mean corpuscular volume of 76.4 fL (RI 79–99 fl), normal hemoglobin of 14.2 g/dL (RI 13.5–17.0 g/dL), and red blood cell distribution width of 14.6% (RI 11.5%–15.0%). Iron studies were not performed.

Initial hemoglobinopathy evaluation was performed by high performance liquid chromatography (HPLC) on a BioRad Variant II system using the β-thalassemia short program. Comparable to an Hb S trait individual (Fig. 1A), HPLC demonstrated a peak consistent with HbS (retention time 4.39 min, area 31.5%) (Fig. 1B), consistent with the positive sickle solubility screen. In addition, presuming an Hb S trait state without history of transfusion, a concurrent alpha thalassemia and/or iron deficiency would be considered due to the lower-than-expected Hb S concentration for Hb S trait as well as borderline abnormal red blood cell counted blood cell count and mean corpuscular volume. However, a second peak was identified in the P3 region (retention time 1.59 min, area 59.4%), indicating the presence of a likely second beta-globin variant. Based on retention times for beta globin variants, a differential for this variant included: Hb Cowtown, Hb Andrew Minneapolis, Hb Malmo, Hb Little Rock, Hb Camden, Hb Riyadh, and Hb Oloumac (1). Capillary zone electrophoresis (CE) using a Sebia CAPILLARYS 2 FLEX PIERCING system was performed for confirmation, which zoned the Hb S peak at migration position 212 (similar to Hb S trait individual in Fig. 2A) and second peak in zone 11, with migration position at 124 (Fig. 2B). Based on these correlations, the closest matches for the second beta globin chain variant included Hb Andrew Minneapolis, Hb Fannin-Lubbock I, Hb New York, and Hb Yagamata (2). Overall, based on these HPLC and CE findings, the Hb variant that may be predicted is Hb Andrew Minneapolis, although it has been typically reported at closer to 40% in trait state (1, 2).

The Sebia software uses a significant Hb A peak as an anchor to allow zoning for most samples (3). The Sebia software can zone when Hb A is absent if significant quantities of both Hb F and Hb A2 are present (3). Despite this improvement, based on the experience of our laboratory and published studies, there is still a possibility for misclassification of hemoglobin variants using this technique (3). Hence, we further investigated by diluting this patient's specimen with normal blood from a different patient (Fig. 2C). Indeed, the abnormal peak was notably different, now appearing in zone 10 with migration position at 135. Presuming this to be a more accurate CE zoning of this variant, a new differential would be encountered, with Hb Camden as the most likely beta globin chain variant, which has been reported in trait states at greater than 50% (1, 2). Given the uncertainty of CE zoning and varied differentials, the case was sent out to our reference laboratory for additional confirmation. Their evaluation cascade included similar techniques as our laboratory as well as isoelectric focusing and intact globin chain mass

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spectrometry. Isoelectric focusing was performed using standard techniques and Schneider-Barwick ratios to measure the band migration pattern corresponding to a variant while the mass to charge ratio from mass spectrometry was utilized to assess the amino acid change in the globin chains. These protein-based characterizations from the various methods were collectively compared to a database to confirm the hemoglobin variant, which, in this case, was consistent with hemoglobin variants of Hb S and Hb Camden.

QUESTIONS TO CONSIDER
• What are the possible considerations for lower-than-expected Hb S concentration?
• How does Sebia CE achieve zoning of hemoglobin fractions when Hb A is absent/present in small • quantities?
• What might be considered as best practice when classifying hemoglobin variants that lack Hb A using • Sebia CE?

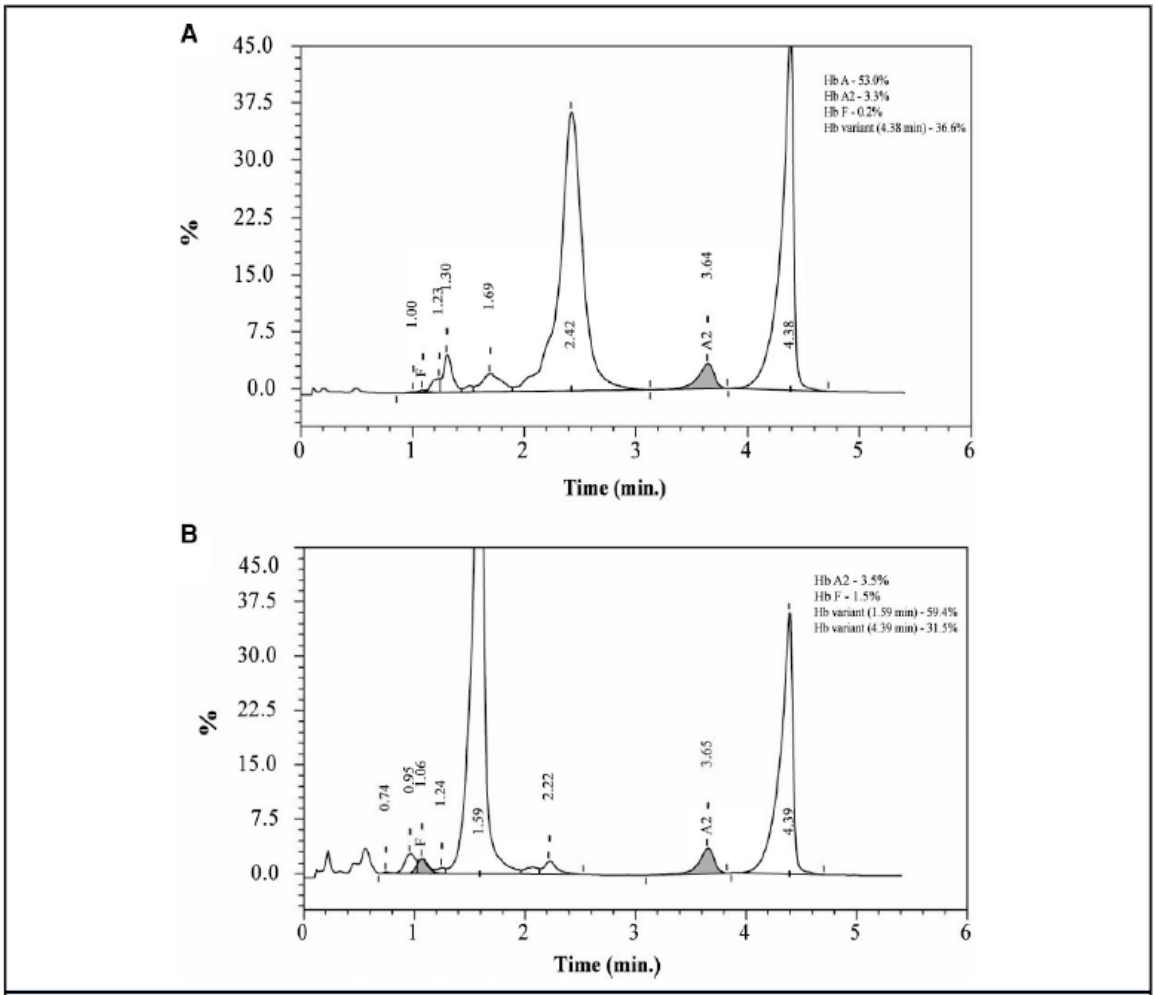

Fig. 1. (A), HPLC showing variant peak in the S window (RT 4.38 min) in an Hb S trait individual; (B), Variant peaks are identified in P3 (RT 1.59 min) and S (RT 4.39 min) windows in Hb S Camden. Abbreviations: RT, retention time.

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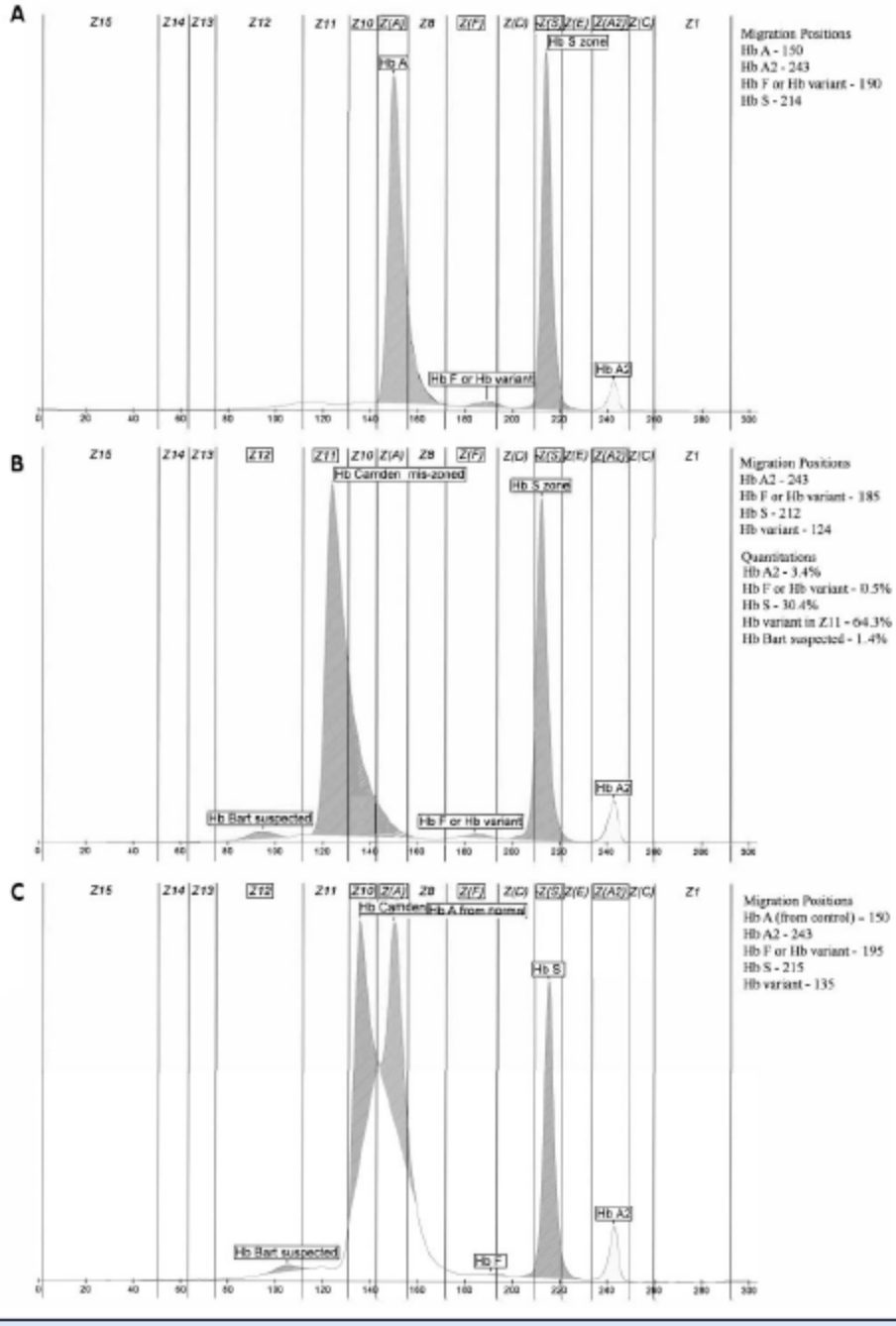

Fig. 2. (A), Capillary zone electrophoresis of an Hb S trait individual showing Hb S variant in Zone 8 at migration position 214; (B), Compound heterozygous Hb S Camden showing a variant peak that is mis-zoned in Zone 11 at migration position 124 and confirming an Hb S variant in Zone S at migration position 214; (C), Mixing with 1:1 blood control containing Hb A and therefore zoning based on Hb A shifts the variant peak accurately to Zone 10 at migration position 135, thus identifying Hb Camden accurately. Labels for the peaks were designated by the software except for "Hb Camden mis-zoned," "Hb Camden," and "Hb A from normal" peaks, which were labeled by our laboratory.

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Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the September 2023 issue of *Clinical Chemistry*. To view the case and comments online, go to https://academic.oup.com/clinchem/issue/69/9 and follow the link to the Clinical Case Study and Commentaries.

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