Unexpected Hemoglobin A_{1c} Results

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[2]Nonstandard abbreviations: Hb, hemoglobin; CE-HPLC, cation-exchange high performance liquid chromatography; Hb S, sickle cell Hb..

CASE

A 52-year-old woman with a medical history of hepatitis B, hyperlipidemia, hypertension, anemia, and depression presented to the internal medicine clinic for a routine visit. Laboratory tests 3 months previously had revealed an impaired fasting glucose concentration of 5.9 mmol/L (106 mg/dL) [reference interval, 3.9 –5.6 mmol/L (70–100 mg/dL)]. Therefore, a hemoglobin (Hb)[2] A_{1c} analysis was performed. The initial Hb A_{1c} evaluation by cation-exchange HPLC (CE-HPLC) (Hb A_{1c} Program on the VARIANT IITURBOLink System; Bio-Rad Laboratories) showed an Hb A_{1c} value of 115.8% (reference interval, 4.0%–6.0%) (Fig. 1). In an effort to determine if the unusual Hb A_{1c} result was due to potential hemoglobinopathies, we performed an Hb variant analysis with the Bio-Rad VARIANT CE-HPLC β-Thalassemia Short Program. The analysis revealed the absence of Hb A and the presence of sickle cell Hb (Hb S) (37.4%), along with normal Hb A_2 (3.2%) and Hb F (<1.0%) (Fig. 2). Also evident was another large peak (53.0%) that eluted earlier than Hb A, which we called P2. This study suggested the presence of an Hb variant with a chromatographic retention time virtually identical to that of Hb A_{1c}, in addition to Hb S (Figs. 1 and 2). A subsequent Hb electrophoretic analysis at pH 6.0 (QuickGel Acid; Helena Laboratories) identified Hb S and another abnormal band with a mobility similar to Hb F (not shown).

PATIENT FOLLOW-UP

To identify the Hb variants, we investigated DNA sequences corresponding to the patient's_β-globin genes. This analysis identified a substitution at codon 6 [GAG to GTG (Glu to Val)] on one allele, corresponding to Hb S, and a substitution at codon 1 [GTG to GCG (Val to Ala)] on the other allele, corresponding to Hb Raleigh. The presence of these hemoglobinopathies suggested that the spuriousHbA_{1c} result obtained with the CE-HPLC method was due to the elution of Hb Raleigh, which has a retention time similar to that of Hb A_{1c}.We evaluated the Hb A_{1c} result with a turbidimetric inhibition immunoassay (Dimension® Clinical Chemistry System; Siemens) and obtained an Hb A_{1c} value of 4.1%, which was not consistent with the impaired fasting glucose concentration of 5.9 mmol/L (106 mg/dL).

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Peak name	Calibrated area, %	Area, %	Retention time, min	Peak area
A_{1a}	—	0.4	0.106	6826
A_{1b}	—	4.6	0.213	76457
A_{1c}	115.8*	—	0.405	816032
P3	—	1.3	0.770	21144
Variant window	—	4.2	0.860	70124
S window	—	40.2	0.920	665722
*Value outside of expected range			Total area:	1656306

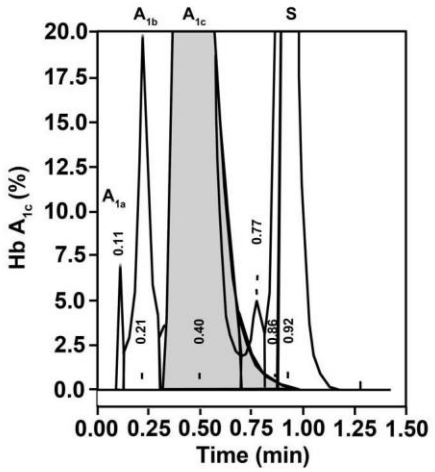

Fig. 1. CE-HPLC chromatogram for Hb A_{1c} analysis.
Hb S and an aberrant Hb A_{1c} value of 115.8% represented the predominant Hb peaks in the chromatogram.

Analyte ID	Percent	Time, min	Area
P1	1.2	0.75	33627
F	0.5	0.94	16262
P2	53.0	1.30	1542147
A_0	2.7	2.28	79887
Unknown 1	1.6	2.50	47682
A_2	3.2	3.68	108007
S window	37.4	4.45	1088414

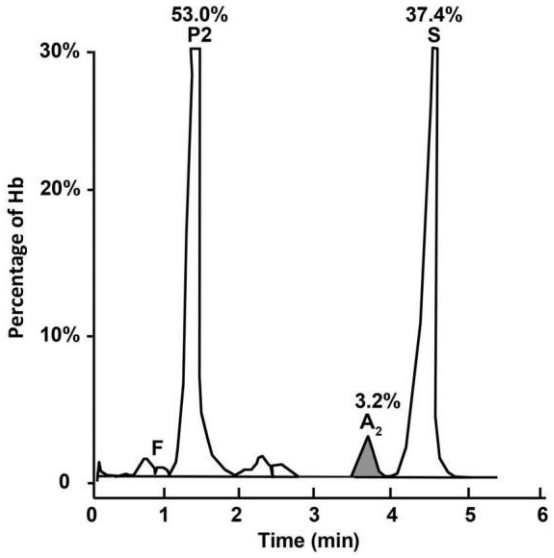

Fig. 2. Chromatogram of Hb variants analysis with the CE-HPLC β-Thalassemia Short Program.
Hb S (37.4%), wild-type Hb A_2 (3.2%), and Hb F (<1.0%) were identified, but Hb A was not detected. A large peak, which we designated P2, was detected at 53.0%.

Questions to Consider
• What are the various types of methods used for measuring Hb A_{1c}?
• How do Hb variants interfere with each of these Hb A_{1c} methods?
• What actions should be taken when a spurious Hb A_{1c} result is present?

Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the February 2011 issue of *Clinical Chemistry*. To view the case and comments online, go to http://www.clinchem.org/content/vol57/issue2 and follow the link to the Clinical Case Study and Commentaries.

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