Letter to the Editor and Authors' Response: Chromatographic Measurements of Hemoglobin A_2 in Blood Samples Containing Sickle Hemoglobin

Letter to the Editor

We would like to comment on the article by Shokrani et al [1], published in the April 2000 issue of the *Annals of Clinical & Laboratory Science.*

The authors begin their abstract by stating that HbA_2 measurements aid in the differential diagnosis of sickle cell anemia from sickle-beta-thalassemia. This, unless meant for the analysis of families, is incorrect. The HbA_2 value is of no diagnostic value at all in the differential diagnosis between HbS/S homozygosity and HbS/beta-thalassemia compound heterozygosity. Furthermore, the "beta-thal short column kit" (Bio-Rad) used by the authors is designed for a reliable determination of the HbA_2 level on the HPLC Variant in the absence of HbS. In the presence of HbS, the glycated products of the HbS fraction will variably overlap with the HbA_2 peak (Figs. 1 and 2, shown in the article) and result, therefore, in the elevated HbA_2 values reported by the authors.

Glycated Hb, comprising 3% to 7% of total Hb, is normally present also in fresh blood. The HbA_2 values reported by the authors are increased by the variable presence of glycated HbS and have no diagnostic significance for hemoglobinopathy analysis, except perhaps for an indication of the (frequently) coexisting alpha-thalassemia when the HbA_2 is lower than 2.5%.

The Variant HPLC analyses cannot differentiate between HbS/S and HbS/beta-zero-thalassemia, if done examining the proband only. First of all, one should be sure about the non-transfused status of the patients (this detail is not mentioned by the authors!). When HbS is present in combination with a mild beta+-thalassemia, a small amount of HbA could be present on HPLC and be a diagnostic indication, but no HbA will be measurable in non-transfused HbS/S or HbS/beta-zero-thalassemia patients. In these cases, the HbA_2 level will be normal or slightly elevated, mostly due to the contamination of the HbA_2 fraction with HbS (glycated) derivatives, depending upon the determination methods. The first indication for HbS/beta-thalassemia will be the presence of microcytic hypochromic parameters, which could also be present in iron depletion or in cases of sickle cell disease in combination with alpha-thalassemia.

Conclusive data are obtained by the globin chain synthesis determination, thus by the measurement of the ratio between the synthetic expression of the betaA+betaS/alpha chains [2]. More straightforward is the direct DNA sequencing of a PCR product containing the first exon of the beta globin gene, which will reveal the cd6 GAG>GTG mutation of the HbS mutant in heterozygous or in homozygous form, respectively.

We agree with the conclusion of the authors that elevated HbA_2 levels measured on the Variant in the presence of HbS must be considered normal. We also agree with the statement that family history is an important element, but even more with the fact that carrier analysis in the parents, if available, may allow the diagnosis in the simplest way.

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The Authors' Response

Hb A_2 measurements alone are not of diagnostic value, but we restate that they aid in the differential diagnosis of sickle cell disease from sickle-β-thalassemia. HbA_2 values are increased in sickle-β-thalassemia (3% to 6%) when compared to sickle cell anemia (2% to 4%) [1]. As we stated, the diagnosis of sickle cell trait, sickle-β-thalassemia, or sickle cell anemia should include family studies and clinical history, since this information may be essential in establishing the diagnosis.

We reported that Hb A_2 values quantitated by the HPLC Variant β-thalassemia Short Program (BioRad Laboratories Inc., Hercules, CA) for sickle cell disorders overlap with those described for β-thalas-semia. Therefore, HbA_2 values determined by this method in samples containing sickle hemoglobin may not be accurate due to falsely elevated HbA_2. The manufacturer states that minor components of HbS and other Hb variants eluting after HbA_2 may co-elute with this Hb, resulting in falsely elevated HbA_2, but does not say that the method should not be used to quantitate HbA_2 in the presence of HbS. We think that it could be used to quantitate HbA_2 in the presence of HbS, as long as the samples are relatively fresh, and that there is a reference range for HbA_2 in the presence of sickle Hb. Our paper provides a reference range for HbA_2 values quantitated by HPLC in sickle cell trait, sickle cell anemia, and HbSC disease. Also, our findings should make hemoglobinopathy screening laboratories aware that HbA_2 determinations by HPLC (BioRad) could be falsely elevated in samples containing HbS, without necessarily implying the presence of a concomitant β-thalassemia. This observation is of particular importance, as more hemoglobinopathy laboratories are switching from the classical electrophoretic systems to the HPLC Variant system (BioRad) for screening of hemoglobinopathies and quantitation of HbF and HbA_2.

In the presence of abnormal β-globin chains, there is increased α-δ-chain interaction, and, in addition, HbS adducts, which include not only glycated HbS, but other post-translational modifications of HbS. Both of these factors contribute to the increased value of HbA_2 in sickle cell disease. Glycated Hb alone does not account for the increase in HbA_2 values [2]. Glycated hemoglobins are present in fresh blood (3% to 7%), however, sample aging contributes to an increased value of glycated Hbs over time [3]. HbA_2 is also increased in sickle cell disease with co-existing α-thalassemia (as determined by microcolumn chromatography) [4], and its value is similar to that found in sickle-β-thalassemia.

We did not mention that the patients in our study were non-transfused. If the HbSS patients had been transfused, their HPLC patterns would have been that of a sickle-β+-thalassemia subject. We did note that samples with a pattern like the one shown in Fig. 1 of the paper were excluded from the study.

We agree that globin chain synthesis ratios (β/α) in reticulocytes are diagnostic [5], as well as DNA sequencing of the β-globin gene [6]. However, these techniques are not available to most hemoglobinopathy screening laboratories. As more laboratories are using HPLC to identify and quantitate hemoglobins, and as proficiency testing programs (eg, by the College of American Pathologists) are taking these HPLC quantitations into account, we consider it important to disseminate our findings of increased HbA_2 in blood samples containing HbS.

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