Interference by Fast Hemoglobin Variants in the Column-Chromatographic Assay for Glycosylated Hemoglobin

To the Editor:

Recently we reported a normal column-chromatographic glycosylated hemoglobin in the presence of a fast hemoglobin variant (putative HbN-Baltimore) (1). Since submission of that manuscript we have learned that another fast hemoglobin, HbH, can increase concentrations of glycosylated hemoglobin (2). Therefore, we undertook further chromatographic and electrophoretic studies of the glycosylation of our fast hemoglobin variant to resolve this apparent conflict.

Our patient's hemolysate was subjected to ion-exchange chromatography for glycosylated hemoglobin determination (Isolab, Akron, OH 44321). The glycosylated hemoglobin eluate was concentrated (Amicon B-15 concentrator; Amicon Corp., Lexington, MA 02173) (2) and subjected to both cellulose acetate and citrate agar electrophoresis (Helena Laboratories, Beaumont, TX 77704).

We believe our results (see Figure 1) show that the glycosylated hemoglobins in our sample have mobility similar to that of their parent hemoglobin at basic pH, but at acid pH these glycosylated hemoglobins have HbF-like mobility in citrate agar. Rahbar and coworkers recognized this for HbA during the late 1960’s (3, 4). The light citrate agar “A” band in the concentrated column eluate represents slight contamination by the fast unglycosylated HbN-Baltimore.

Kruiswijk et al. (2) used cellulose acetate electrophoresis to show contamination of their eluate by unglycosylated HbH. We believe that the fast hemoglobin in their column eluate could have been glycosylated HbH. Citrate agar electrophoresis has been used to measure glycosylated hemoglobin (5) and is analogous to ion-exchange chromatography (6). Hence, we believe the better method to show co-elution of glycosylated HbA and HbH with unglycosylated HbH would have been citrate agar electrophoresis. By this method all glycosylated hemoglobin in the column eluate, including glycosylated HbH, would be expected to have HbF-like (cathodal) mobility while any hemoglobin of HbA-like mobility would represent unglycosylated HbH. We do not, however, disagree with the conclusion of Kruiswijk et al. The increased glycosylated hemoglobin in their case is compatible with contamination by unglycosylated HbH. Furthermore, the insignificant contamination by unglycosylated fast hemoglobin in our case is consistent with their conclusion. Finally, HbH is a faster (more anodal) hemoglobin at basic pH than HbN-Baltimore (7) and therefore might be more likely to falsely increase chromatographic glycosylated hemoglobin values.

References

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Bayes’ Theorem and the Estimation of the Likelihood Ratio

To the Editor:

Recently, Keller and Gesaner (1) suggested that the data base for the estimation of likelihood ratios should be restricted to the zone of overlap. This seems to be attractive, because probabilistic reasoning is necessary in this range only. However, final odds cannot be obtained unless the initial odds are also estimated for the same population (i.e., in the range of overlap). This requirement makes Keller and Gesaner’s approach impractical.

The calculation of likelihood ratios implies the estimation of two condi-

References