the Dunnett multiple comparison test) in the test group (Fig. 1A), whereas they did not change significantly in the control group (Fig. 1B). The reduction was highest at $t_2$ when most of calcium was absorbed by the gut. The results obtained indicate that $\beta$-CTx in the serum is able to detect the perturbation of calcium homeostasis induced by calcium loading. The dynamics of the changes observed strongly confirm the view that osteoclasts are involved in the short-term error correction mechanism of plasma calcium homeostasis (5). It is in fact conceivable that the decrease in serum $\beta$-CTx concentrations after calcium loading could be ascribed only to the inhibition of osteoclast activity induced by the calcemic increment: any possible calcium-related changes in the degradative pattern of collagen are unlikely because of the short-term nature of the experiment, and further degradation of cross-links and related peptides in the serum rather than their relevant basal values ($r = -0.90; P <0.0001;\text{Fig. 1D}$).

By dividing the subjects taking calcium according to basal bone turnover into two subgroups, one with $t_0$ $\beta$-CTx concentrations higher than the mean value (2901 pmol/L) and the other with concentrations lower than the mean value, we found a highly significant difference ($P <0.0001$, two-tailed Student $t$-test) between the amplitude of the decrements (2904 ± 268 pmol/L vs 862 ± 220 pmol/L, respectively). Two metabolic factors, therefore, appear to modulate the decrement observed in the $\beta$-CTx serum concentration as they did with total Dpd in the urine: one is the amplitude of the perturbation in plasma calcium homeostasis, and the other is the osteoclast activity. Any circadian daylight variations in the $\beta$-CTx concentrations in serum during the experimental time cannot be taken into account to explain the changes observed because serum $\beta$-CTx values did not change significantly in the control group. When the clear-cut difference in the pattern of the changes observed in the subjects taking calcium or not (Fig. 1, A and B) is considered, it can be inferred that serum measurement of $\beta$-CTx may better discriminate parathyroid hormone-dependent (test group) and -independent (control group) changes of osteoclast activity than urinary total Dpd (5).

We therefore conclude that in our experimental conditions, the clinical performance of the serum $\beta$-CTx is comparable to that obtained by total urinary Dpd. In the clinical setting, it could be advantageous to measure the cross-links and related peptides in the serum rather than in the urine, thus avoiding possible bias related to creatinine correction and renal handling.

We thank Dr. C. Nassivera (Cis Diagnostici S.p.A.) for the kind gift of the Serum CrossLaps One Step ELISA assays.

**References**


**Effects of Nine Hemoglobin Variants on Five Glycohemoglobin Methods,** William L. Roberts,1,2 Elizabeth L. Frank,3 Linda Moulton,2 Christine Papadea,4 Jimmie K. Noffsinger,4 and Ching-Nan Ou 5

Most studies of the effects of variant hemoglobins (Hbs) on specific glycohemoglobin (gHb) methods have been case reports of a single variant Hb and one or two analytical methods (1–4). Few studies have systematically examined multiple Hb variants with several widely used analytical methods. In this report, we describe the effects of nine heterozygous Hb variants on five gHb methods. A boronate affinity method was chosen as the comparative method because it has high specificity for glycated Hb and negligible interference by variant Hbs (5).

Over 10 months, we studied 40 samples with nine variant Hbs; 38 were detected during routine gHb analysis by a cation-exchange method. Two samples (Hb E trait) were identified during routine Hb phenotype analysis. Samples were stored at 2–8 °C until analysis within 10 days of collection.

Cation-exchange chromatography was performed on a Variant system with the Hb A1c program (Bio-Rad Labo-
Hb phenotype analysis was performed on all samples using a PVS99 system (Primus). This HPLC system uses a poly-aspartic acid cation-exchange column with a complex nonlinear salt and pH gradient at 40 °C. Rare phenotypes, including Grady, Hope, and Raleigh were identified using a poly-aspartic acid column with a nonlinear salt and pH gradient (6) and comparison with the authentic Hb variants. The effects of variant Hbs on each gHb method are summarized in Table 1. Neither immunoassay method showed significant effects with any of the Hb variants tested. One sample containing Hb Camden trait was investigated. The Variant method poorly resolved Camden from Hb A and underestimated gHb (Fig. 1A).

Eight samples with Hb D Los Angeles trait were investigated. Three different chromatographic patterns were observed for the Variant method. In the first pattern (Fig. 1B), Hb A was resolved from glycated Hb D (Hb D1c) and Hb D. However, the instrument printout included the area of Hb D in the total for Hb A and consequently underestimated gHb. Manipulation of the peak areas either by summing Hb A1c and Hb D1c and dividing by the total area or by dividing the Hb A1c peak area by the Hb A peak area failed to yield results within 1% of the comparative method. In the second pattern (Fig. 1C), Hb D was not resolved from Hb A, with consequent underestimation of gHb. The chromatograms were virtually identical to Fig. 1E and comparison with the authentic Hb variants indicated coelution of Hb J Baltimore and A. Consequently, gHb was underestimated. When gHb was recalculated as the sum of the peak areas of glycated Hbs A and J Baltimore divided by the total area, the value yielded was low. Inspection of the chromatogram revealed that the method of drawing the baseline (an inverted “v”) under the Hb J Baltimore1c and A1c peaks was responsible for an underestimation of the true areas of both of these peaks. Of the 14 Variant results for Hb J Baltimore samples, only 2 showed flat baselines and gHb showed good agreement with the comparative method for all Hb D samples tested.

Two samples from nondiabetic patients with Hb E trait were analyzed. Neither chromatographic method resolved Hb A from Hb E. The Variant method did not resolve Hb A1c from Hb E1c and results agreed well with the comparative method. The A1c 2.2 Plus method resolved Hb A1c from Hb E1c, but did not resolve Hb A from Hb E, with consequent underestimation of gHb.

Six samples containing Hb G Philadelphia trait were analyzed. The Variant method produced three chromatographic patterns resembling those seen for Hb D in Fig. 1, B–D. gHb was underestimated. The A1c 2.2 Plus method overestimated gHb compared with the comparative method because of coelution of Hb A1c and Hb G1c, whereas Hb A and G were resolved.

Two samples containing Hb Grady trait were evaluated. The chromatograms were virtually identical to Fig. 1C. However, the Variant results agreed with the comparative method without correction. The A1c 2.2 Plus showed good agreement with the comparative method.

Five samples containing Hb Hope trait were evaluated. Hb Hope comigrated with Hb A1c in the Variant method, with a large overestimation of gHb. The A1c 2.2 Plus method resolved Hb Hope from Hb A1c and Hb A (Fig. 1E) and yielded gHb values comparable to the comparative method. Fourteen samples containing Hb J Baltimore trait were evaluated. A Variant chromatogram (Fig. 1F) showed a peak eluting just before Hb A1c, which presumably is Hb J Baltimore1c. A single peak at 1.56 min indicated coelution of Hb J Baltimore and A. Consequently, gHb was underestimated. When gHb was recalculated as the sum of the peak areas of glycated Hbs A and J Baltimore divided by the total area, the value yielded was low. Inspection of the chromatogram revealed that the method of drawing the baseline (an inverted “v”) under the Hb J Baltimore1c and A1c peaks was responsible for an underestimation of the true areas of both of these peaks. Of the 14 Variant results for Hb J Baltimore samples, only 2 showed flat baselines and gHb showed good agreement with the comparative method for all Hb D samples tested.

Two samples from nondiabetic patients with Hb E trait were analyzed. Neither chromatographic method resolved Hb A from Hb E. The Variant method did not resolve Hb A1c from Hb E1c and results agreed well with the comparative method. The A1c 2.2 Plus method resolved Hb A1c from Hb E1c, but did not resolve Hb A from Hb E, with consequent underestimation of gHb.

Six samples containing Hb G Philadelphia trait were analyzed. The Variant method produced three chromatographic patterns resembling those seen for Hb D in Fig. 1, B–D. gHb was underestimated. The A1c 2.2 Plus method overestimated gHb compared with the comparative method because of coelution of Hb A1c and Hb G1c, whereas Hb A and G were resolved.

Two samples containing Hb Grady trait were evaluated. The chromatograms were virtually identical to Fig. 1C. However, the Variant results agreed with the comparative method without correction. The A1c 2.2 Plus showed good agreement with the comparative method.

Five samples containing Hb Hope trait were evaluated. Hb Hope comigrated with Hb A1c in the Variant method, with a large overestimation of gHb. The A1c 2.2 Plus method resolved Hb Hope from Hb A1c and Hb A (Fig. 1E) and yielded gHb values comparable to the comparative method. Fourteen samples containing Hb J Baltimore trait were evaluated. A Variant chromatogram (Fig. 1F) showed a peak eluting just before Hb A1c, which presumably is Hb J Baltimore1c. A single peak at 1.56 min indicated coelution of Hb J Baltimore and A. Consequently, gHb was underestimated. When gHb was recalculated as the sum of the peak areas of glycated Hbs A and J Baltimore divided by the total area, the value yielded was low. Inspection of the chromatogram revealed that the method of drawing the baseline (an inverted “v”) under the Hb J Baltimore1c and A1c peaks was responsible for an underestimation of the true areas of both of these peaks. Of the 14 Variant results for Hb J Baltimore samples, only 2 showed flat baselines and gHb showed good agreement with the comparative method for all Hb D samples tested.

Two samples from nondiabetic patients with Hb E trait were analyzed. Neither chromatographic method resolved Hb A from Hb E. The Variant method did not resolve Hb A1c from Hb E1c and results agreed well with the comparative method. The A1c 2.2 Plus method resolved Hb A1c from Hb E1c, but did not resolve Hb A from Hb E, with consequent underestimation of gHb.
values that agreed closely with the comparative method after recalculation. Inverted “v” baselines were seen for the other 12. A chromatogram obtained by the A1c 2.2 Plus method (Fig. 1G) showed an extra peak, which corresponded to Hb J Baltimore1c. Hb A and J Baltimore coeluted, with consequent underestimation of gHb.

One sample with Hb Raleigh trait was investigated. This variant Hb comigrated with Hb A1c in both the Variant and A1c 2.2 Plus methods (Fig. 1H), leading to a large overestimation of gHb. The DCA 2000 and comparative methods agreed.

One Hb Russ sample was evaluated. This variant eluted after Hb A in both the Variant (Fig. 1I) and A1c 2.2 Plus methods. The elution position of Hb Russ1c was not

Fig. 1. Chromatograms of selected variant Hbs.
The Hb A1c peak is shaded. (A), Variant chromatogram of Hb Camden trait. (B–D), Variant chromatograms of Hb D Los Angeles trait. (E), A1c 2.2 Plus chromatogram of Hb Hope trait. (F), Variant chromatogram of Hb J Baltimore trait. (G), A1c 2.2 Plus chromatogram of Hb J Baltimore trait. (H), A1c 2.2 Plus chromatogram of Hb Raleigh trait. (I), Variant chromatogram of Hb Russ trait.
evident. Nevertheless, gHb was accurately estimated by both methods.

Cation-exchange gHb methods can be affected by variant Hbs in one of three ways: (a) Hb A1c may be resolved from the Hb variant, whereas Hb A is poorly resolved or not resolved at all from the Hb variant, leading to an underestimation of gHb. Several of the Hb variants tested, including D Los Angeles, G Philadelphia, and J Baltimore showed this limitation with the Variant method. However, only Hb E and J Baltimore have this limitation with the A1c 2.2 Plus method. (b) The Hb variant can comigrate with Hb A1c, leading to an overestimation of gHb. Hb Raleigh had this effect in the Variant method, and Hb Raleigh had this effect in both cation-exchange methods. These Hb variants generally account for 35–45% of the total Hb and therefore cause gHb to be >20%, a concentration seldom seen in diabetic patients. (c) The Hb variant A can comigrate with Hb A1c, leading to its overestimation if the Hb variant is resolved from Hb A. This seems to occur for G Philadelphia with the A1c 2.2 Plus system.

The effects of variant Hb traits on Hb A1c results produced by the Variant and DCA 2000 methods had been examined previously (7). These investigators concluded that if appropriate calculations were performed, samples containing Hb D and Hb J Baltimore traits could be accurately analyzed using the Variant system. In contrast, we found a great deal of variability in the chromatograms generated by the Variant system with these two Hb variants. Rarely, gHb could be correctly estimated by summing the peak areas of the glycated Hb A and Hb variant and dividing by the total area. However, in most cases this approach did not work.

The practice of correcting results for samples containing Hb variants by summing the peak areas of glycated A and the glycated variant and then dividing by the total area can be problematic not only when chromatographic resolution is poor, as discussed above, but also when the specific variant is not known. This is best illustrated for samples containing Hb Grady trait.

The two immunoassay methods evaluated produced results that agreed well with the boronate affinity comparative method. Only Hb Raleigh has an amino acid substitution in the six N-terminal amino acids of the β chain. It previously has been shown that the presence of Hb C or S trait, both of which have substitutions at position six of the β chain, can affect the accuracy of some immunoassay results (8). Other variant Hbs with substitutions or deletions in the first six amino acids of the β chain, including Deer Lodge, Fukoka, C_Makassaar, Leiden, Long Island, Machida, Marseille, Okayama, Raleigh, South Florida, and Warwickshire have the potential to adversely affect the accuracy of immunoassay gHb methods (9). Herein, the DCA 2000 result (5.4%) was within 1% of the boronate affinity result (6.3%) for one sample containing Hb Raleigh. A previous study of Hb Raleigh, whose β-chain NH2 terminus is acetylated, suggested that both immunoassay methods might underestimate glycated Hb Raleigh (and that boronate affinity methods may underestimate mean blood glucose because the acetylated N-terminal amino acid cannot be glycated) (10).

In conclusion, several variant Hbs can produce variable interferences with both of the cation-exchange chromatography gHb methods that we evaluated. The DCA 2000 and Tina-quant methods agreed well with boronate affinity chromatography.

A DCA 2000 Analyzer and reagents were provided by Bayer Corporation. Tina-quant HbA1c II reagents were provided by Roche Diagnostics.

References


Preliminary Evaluation of the Vitros Eci Cardiac Troponin I Assay, Fred S. Apple,* Brenda Koplen, and MaryAnn M. Murakami (Hennepin County Medical Center, Clinical Laboratories MC 812, 701 Park Ave., Minneapolis, MN 55425; *author for correspondence: fax 612-904-4229, e-mail fred.apple@co.hennepin.mns.us)

The recently published standards of practice for the use of cardiac marker testing for ruling in and ruling out acute myocardial infarction (AMI) recommend the implementation of cardiac troponin I (cTnI) or T (cTnT) as appropriate markers (1). The purpose of this preliminary study was to analytically and clinically evaluate the Ortho-Clinical Diagnostics (Rochester, NY) Vitros Troponin I immunodiagnostic assay.

The Vitros Eci system uses reagents containing biotinylated monoclonal anti-cTnI antibody and goat polyclonal anti-cTnI antibody labeled with horseradish peroxidase. After an 8-min incubation for reagents and sample in a well precoated with streptavidin, the well is washed and