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 Variant method. However, in most cases this approach did not work. (b) The Hb variant can comigrate with HbA1c, leading to an overestimation of gHb. Hb Hope 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 A1c 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-Makassar, 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 Mary/Ann 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.mn.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-cTnT 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
a signal reagent containing horseradish peroxidase substrates is added to detect peroxidase bound to the well by utilizing an enhanced chemiluminescence reaction (2).

The imprecision (as the CV, %) within the same calibration performed according to NCCLS guidelines (3) showed the following results: between-run (n = 80) means over 31 days on 20 occasions were 0.347 μg/L (10%), 0.768 μg/L (5.9%), 4.10 μg/L (4.3%), and 15.5 μg/L (3.2%). The analytical sensitivity, determined using 2 SD after 20 replicates of the zero calibration material were measured, was 0.018 μg/L. The lowest concentration giving rise to a between-assay CV ≤10% was 0.351 μg/L. Deming relationships, obtained from 36 fresh plasma samples across the linear range (0–100 μg/L) of the Vitros cTnI assay compared with the Dade-Behring RxL cTnI assay, showed the following: Vitros cTnI = 0.556 Dade cTnI + 0.039; r = 0.972. The 95% confidence intervals for slope and intercept were 0.509–0.602 and −0.394 to 0.472, respectively. The difference plot (Exstat Ver. 9; DDU Software) shown in Fig. 1A demonstrates a proportional, increasing negative bias across the range of concentrations tested for by the ECi cTnI assay compared with the Dade RxL cTnI assay. The bias is likely a result of lack of standardization between cTnI assays (4). Deming regression analysis for the Vitros cTnI assay for fresh plasma samples (n = 50) compared with the same frozen, thawed plasma samples showed no significant differences (frozen cTnI = 0.965 fresh cTnI − 0.005; r = 0.997) over a cTnI range of 0–11.3 μg/L. Deming regression analysis between paired fresh serum and plasma (heparinized) samples showed a bias for plasma as follows: plasma Vitros cTnI = 0.760 serum Vitros cTnI − 0.201; r = 0.998 (n = 17). Based on 200 apparently healthy male and female blood donors, the upper reference 97.5th percentile was 0.1 μg/L in serum and 0.08 μg/L in plasma samples. Fig. 1B shows a comparison of ROC curves for the ECi and RxL assays determined from 74 chest pain patients admitted to rule in or rule out AMI. A modified WHO criterion, using a cTnI clinical decision cutpoint at 0.8 μg/L (Dade Behring RxL assay) for the biochemical criterion (predetermined at Hennepin County Medical Center), was used (5). Peak cTnI values, from the three to four plasma samples obtained serially within 24 h of presentation to the hospital, were used to construct the ROC curves. Thirty percent (22 of 74) of patients were diagnosed with AMI. The ECi cTnI cutoff of 0.40 μg/L demonstrated a sensitivity of 100% and a specificity of 79%. In comparison, the RxL cTnI cutoff of 0.55 μg/L demonstrated a sensitivity of 100% and a specificity of 77%. There were no statistical differences in the areas under the ROC curves between assays (0.9038 for ECi; 0.9008 for RxL). Patients with clinical diagnoses involving myocardial damage, including acute coronary syndromes (unstable angina), congestive heart failure, and cardiac surgery, but excluding AMI, were responsible for the 77% and 79% specificities of both cTnI assays at 100% sensitivities. Outcome studies involving risk assessment, however, were not part of this study design.

In summary, the Vitros ECi Troponin I immunodiagnostic assay demonstrated acceptable analytical performance. The impression around the upper reference cutpoints was clinically acceptable (<10%). The 97.5th
percentile was substantially different (four- to fivefold higher) than the analytical sensitivity, allowing for acceptable clinical specificity. Furthermore, there was excellent clinical concordance between the Vitros ECi and Dade RxL cTnI assays. The cTnI values obtained with the ECi were approximately one-half of those obtained with the RxL cTnI, probably because of the lack of antigen and antibody standardization between assays (4). Thus, the Vitros ECi Troponin I immunodiagnostic assay appears to be an acceptable alternative for monitoring plasma or serum cTnI for ruling in and ruling out AMI. As for risk stratification in acute coronary syndrome patients (6), additional clinical evaluation studies will be necessary to validate its applicability in clinical practice.

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References

Semiautomated Method for Determination of Cystine Concentration in Polymorphonuclear Leukocytes, Anna Pastore,1 Anna Lo Russo,1 Marcella Greco;2 Gianfranco Rizzoni,1 and Giorgio Federici2 (1 Laboratory of Clinical Biochemistry and 2 Division of Nephrology and Dialysis, Children’s Hospital and Research Institute “Bambino Gesù”, Piazza S. Onofrio 4, 00165 Rome, Italy; * author for correspondence: fax 39-06-68592210, e-mail Federici@obg-irccs.rm.it)

Cystinosis is an autosomal recessive disease caused by impaired transport of cystine across lysosomal membranes. The subsequent lysosomal storage of the poorly soluble cystine produces crystal formation and cellular damage in many tissues. The earliest involvement occurs in the renal tubules and causes Fanconi syndrome, with polyuria, dehydration, acidosis, rickets, and failure to thrive. In untreated cystinosis, the progression of renal glomerular dysfunction leads to uremia and death by 9–10 years of age unless dialysis or renal transplantation is initiated (1). The therapy of nephropathic cystinosis involves treatment with the cystine-depleting agent cysteamine (2).

The most direct diagnostic method for cystinosis is measurement of leukocyte cystine content by an Escherichia coli cystine-binding protein assay. This assay is time-consuming and involves competition between nonradioactive cystine and [14C]cystine for a cystine-binding protein. The protein-bound radioactivity is then trapped on nitrocellulose filters and is inversely correlated to nonradioactive cystine (3).

Here we present a method for measuring the cystine content of polymorphonuclear (PMN) leukocytes that is based on HPLC with fluorescence detection (FD) (4, 5); this method is reproducible, sensitive, and requires no radioactive reagents.

Blood (4.5 mL) was drawn by venipuncture and was collected into a heparin-containing tube. PMN leukocyte separations were carried out as described by Smolin et al. (6). After sonication of the PMN leukocytes three times for 2 s (each) in 0.1 mL of 0.1 mol/L phosphate buffer, pH 7.2, containing 5 mmol/L N-ethylmaleimide (NEM), 50 μL of 120 g/L sulfosalicylic acid was added, and the cystine content in the acid-soluble fraction was determined. The protein pellet was then dissolved in 150 μL of 0.1 mol/L NaOH, and the protein concentration was determined with the BCA protein assay (Pierce).

The derivatization and chromatography procedures were performed, with little modification, as reported previously (4). Briefly, the autosampler collected 3 μL of 4 mol/L NaBH₄, 2 μL of 2 mmol/L EDTA-dithiothreitol, 1 μL of 1-octanol, and 2 μL of 1.8 mol/L HCl and placed the mixture in the derivatization vial containing 10 μL of sample. After the mixture was incubated for 3 min, 10 μL of 1.5 mol/L N-ethylmorpholine buffer, pH 8.0, 40 μL of distilled water, and 2 μL of 25 mmol/L bromobimane were added. After an additional 3-min incubation, 4 μL of acetic acid was added, and 40 μL of this mixture was injected into the column.

Known concentrations of cystine were added to samples. The concentrations in samples with added cystine were determined in five replicates, and analytical recoveries were calculated. The intraassay precision was obtained by analyzing 10 replicates of the biological samples on the same day. The interassay precision was determined by analyzing the same biological samples on 10 different days over 1 month.

Calibration curves for cysteine (0.3–10 μmol/L) were prepared in duplicate by diluting the stock solutions with 0.1 mol/L HCl containing 100 μmol/L dithiothreitol. The linearity of the assays was also studied in the following range: 0–10 μmol/L cysteine. The limit of detection, defined as the concentration that produces a signal-to-noise ratio >3, was ~0.4 pmol in the assay.

In a typical HPLC-FD chromatogram of a sample from a healthy subject (Fig. 1A), the retention time for cysteine-S-bimane was 3.7 min. The cysteine peak at 3.7 min in Fig. 1A was given by a sample concentration of 0.08 nmol half-cysteine/mg protein. In a typical chromatogram from an individual with cystinosis (Fig. 1B), the cysteine-