Performance of the Roche Second Generation Hemoglobin A1c Immunoassay in the Presence of Hb-S or Hb-C Traits

Jude M. Abadie and Angela A. Koelsch
Pathology Department, Walter Reed Army Medical Center, Washington, District of Columbia

Abstract. Blood HbA1c determination is a powerful tool for the evaluation and management of patients with diabetes mellitus. Many HbA1c analytical methods demonstrate bias in samples from patients with hemoglobinopathies. This study evaluated the analytical performance of Roche Diagnostics’ 1st and 2nd generation HbA1c assays in patients with or without hemoglobinopathies whose HbA1c levels were elevated or normal, respectively. Boronate-affinity high performance liquid chromatography (HPLC) served as the reference method. Whole blood samples were collected from 80 patients with HbS or HbC whose group mean HbA1c value was elevated and also from 80 patients without hemoglobinopathy whose HbA1c values were in the well-controlled range. Each sample was assayed for HbA1c by the Primus boronate-affinity HPLC technique and by Roche’s 1st and 2nd generation immunoassays using a Cobas Integra 800 analytical system. Results by the HPLC technique were compared with the results of both Roche assays by linear regression and Bland-Altman analysis. The 1st and 2nd generation assays yielded regression lines and correlation values vs HPLC assay of y = 1.43x - 1.59; R² = 0.83, and y = 0.94x + 0.10; R² = 0.92, respectively, in the 80 patients with hemoglobinopathies. The mean difference and the ±2SD range were greater in the 1st than in the 2nd generation assay (2.68, ±2.07 vs -0.54, ±0.86, respectively). The 2nd generation assay also showed better performance than the 1st generation assay in samples from the 80 patients without hemoglobinopathy. In conclusion, this study validates the accuracy of Roche’s 2nd generation assay, which is substantially improved over Roche’s 1st generation HbA1c assay.

Keywords: hemoglobin A1c, hemoglobinopathies, HbS, HbC, diabetes mellitus, HbA1c immunoassay

Introduction

Accurate control of blood glucose can ameliorate much of the morbidity and mortality associated with diabetes mellitus [1]. This clinical need has generated efforts to improve the accuracy of hemoglobin A1c (HbA1c) testing. The National Glycohemoglobin Standardization Program sets guidelines and certifies methods for HbA1c assay so that long-term glucose control of diabetic patients can be reliably evaluated and managed. Many assays for glycohemoglobin (gHb) are available, based on physical and chemical properties, or specific antibody-recognized epitopes.

About 80% of blood HbA1 is a hemoglobin (Hb) tetramer glycated at the N-terminus of the β chain [2]. As defined by the International Federation of Clinical Chemistry (IFCC), HbA1c is Hb that is irreversibly glycated at one or both N-terminal valines of the β chain. Most of the more than 700 identified Hb variants arise from point mutations in the α, β, γ, or δ Hb chains [3]. Studies suggest that >200,000 of the 16 million patients with diabetes in the US require gHb measurement in the presence of hemoglobin variants [4-6]. HbS and HbC are the most common variants, and HbS trait occurs in a significant proportion of diabetic patients who are tested for HbA1c worldwide [7]. In addition to genetic variants of Hb, structural modifications of gHb that result from chemical alterations are often present in diabetic patients [5,6].
Several studies demonstrate significant bias in analytical methods used to measure gHb, and this bias causes inaccurately calculated HbA1c values [8-10]. Inaccurate measurements of HbA1c are especially problematic in patients with genetically or chemically modified Hb [10-13]. HbS and HbC traits are the most clinically significant causes of interferences that lead to inaccurately high HbA1c assessment [6]. The clinical importance of this is especially evident when HbA1c overestimation leads to aggressive glucose management, resulting in more frequent hypoglycemic episodes.

This study was performed at Walter Reed Army Medical Center (WRAMC) to evaluate and compare the analytical performance of the Roche “Tina-quant” 2nd generation HbA1c assay and the Roche “Unimate” 1st generation HbA1c assay in (a) patients without hemoglobinopathy whose HbA1c levels are normal and (b) in patients with hemoglobinopathy (HbS or HbC) whose HbA1c levels are elevated. Using a boronate-affinity HPLC assay as the reference procedure, we evaluated the manufacturer’s claim that the “Tina-quant” 2nd generation assay provides improved accuracy in patients with both a hemoglobinopathy and elevated HbA1c levels.

Materials and Methods

As approved by WRAMC’s Institutional Review Board, this study was composed of 2 different patient groups. The first group consisted of 80 adult African American patients with a hemoglobinopathy (68 HbS trait or 12 HbC trait) who had an elevated group-mean (11.3%) HbA1c value (Group 1). The second group (Group 2) consisted of 80 adult patients without hemoglobinopathy whose HbA1c values were in the reference range (<7.0%)). For both patient groups, blood HbA1c was assayed as part of routine medical care. Hemoglobinopathy status was based on patient history or Hb electrophoresis.

All HbA1c values were determined using 3 different methods according to the manufacturers’ recommendations. The HbA1c assays were: (a) “Unimate” 1st generation assay (Roche Diagnostics), (b) “Tina-quant” 2nd generation assay (Roche Diagnostics), and (c) HPLC via boronate-affinity chromatography (Primus Corporation).

The “Unimate” 1st generation assay, a reagent system for quantitative determination of HbA1c in whole blood, was performed with the Cobas Integra 800 automated analyzer. After hemolysis of the anticoagulated whole blood sample, total Hb and HbA1c values were determined colorimetrically and immunoturbidimetrically, respectively. The (%HbA1c was calculated from the ratio of Hb and HbA1c concentrations [(%HbA1c = HbA1c/Hb) x 198.9 + 1.72]). Monoclonal antibodies (Ab) that are attached to latex particles bind to β-N-terminal HbA1c fragments. A synthetic polymer with multiple β-N-terminal standard HbA1c copies is used to agglutinate remaining free Ab. The turbidity change measured at 552 nm is inversely related to the concentration of bound glycopeptides.

The “Tina-quant” 2nd generation assay for quantitative determination of HbA1c in whole blood was also performed with the Cobas Integra 800 automated analyzer. The HbA1c determination is based on turbidimetric inhibition immunoassay (TINA) of hemolyzed whole blood samples. The manufacturer claims the assay detects Hb variants with both HbA1c Ab recognizable regions and glycation at the β-chain N-terminus. HbA1c in the sample reacts with anti-HbA1c Ab, forming a soluble Ag-Ab complex. There is no further complex formation because the HbA1c Ab site is present only once on the HbA1c molecule. Polyhaptens react with excess anti-HbA1c Ab, and the formation of an insoluble Ab-polyhapten complex is turbidimetrically determined. In the hemolyzed blood sample, liberated Hb derivative is measured bichromatically and calculations are performed according to IFCC guidelines as %HbA1c = (HbA1c/Hb) x 87.6 + 2.27.

The Brook Army Medical Center in San Antonio, Texas, performed the HbA1c analyses using the Primus Corporation HPCL analytical system, based on boronate-affinity chromatography. This HPLC system generates the least interference from Hb variants and derivatives [4,14-15]. The method determines total gHb, including HbA1c and ketoamine structures formed on lysines and N-terminal valine residues of both the α and β chains. The m-aminophenylboronic acid, cross-linked to agarose or glass beads, reacts only with cis-diol glucose groups bound to Hb. This forms a reversible 5-member ring complex that subsequently immobilizes the gHb to the column. Sorbitol is used to dissociate the complex and elute gHb [2], which is then measured spectrophotometrically.

The blood samples that were analyzed off-site by HPLC were de-linked to patient information and given by protocol a random sample number with the corresponding HPLC value. The samples were shipped to WRAMC on dry ice and kept frozen until they were analyzed by the “Unimate” and “Tina-quant” assays.

Statistical analyses were performed using SPSS 9.0 software (SPSS Inc., Chicago, IL). Regression analysis by the least squares method was used to generate correlation coefficients and linear equations. Bland-Altman plots were generated using the same software.

Results

Figs. 1 and 2 show the relationships between HbA1c results by boronate-affinity HPLC vs those by the “Unimate” 1st generation and “Tina-quant” 2nd generation assays in 80 adult African American patients with hemoglobinopathy (Group 1). The 2nd generation assay gave much better correlation with the HPLC results (y = 0.94x + 0.10; R² = 0.92) than did the 1st generation assay (y = 1.43x - 1.59;
Fig. 1. Relationship between HbA1c results by boronate-affinity HPLC vs the "Unimate" 1st generation immunoassay in 80 patients with elevated mean HbA1c level and either HbS or HbC traits.

\[ Y = 1.43x - 1.59 \]
\[ R^2 = 0.83 \]
\[ N = 80 \]
\[ \text{Mean} = 12.2 \]
\[ \text{Range} (4.8 - 19.9) \]

Fig. 1. Relationship between HbA1c results by boronate-affinity HPLC vs the "Tina-quant" 2nd generation immunoassay in 80 patients with elevated mean HbA1c level and either HbS or HbC traits.

\[ Y = 0.94x + 0.10 \]
\[ R^2 = 0.92 \]
\[ N = 80 \]
\[ \text{Mean} = 12.2 \]
\[ \text{Range} (4.8 - 19.9) \]
R² = 0.83) in the 80 patients with HbS or HbC whose mean HbA1c value was 12.2%. Bland-Altman difference plots of the same data (Figs. 3, 4) show a much greater mean difference and ±2 SD range with the 1st generation assay compared to the 2nd generation assay (2.68, ±2.07; -0.54, ±0.86), respectively.

In the group of 80 patients without a hemoglobinopathy whose blood HbA1c value was <7.0% (Group 2), the 2nd generation assay also yielded more accurate performance than the 1st generation assay (Fig. 5).

**Discussion**

Our study supports previous reports of decreased Hb variant interference in the “Tina-quant” 2nd generation immunoassay for HbA1c [4,8-9].
study demonstrates improved analytical accuracy in the presence of HbS or HbC traits at significantly elevated HbA1c values. Although manufacturers of some HbA1c point-of-care tests (POCT) claim that their methods have reduced interference from various Hb variants, a recent evaluation of POCT performance demonstrates clinically significant positive interference in samples with HbS trait [11], the most commonly encountered hemoglobinopathy.

Accuracy of HbA1c results is an analytical and clinical concern for the approximately 30 million African Americans >17 yr old [4,9,14,17]. It is estimated that at least 10% of this population has either HbS or HbC trait, as well as a prevalence for diabetes that is almost 10% [18]. The improved accuracy of Roche’s 2nd generation immunoassay, as demonstrated by our results, can lead to better clinical monitoring decisions and improved care for patients with HbS or HbC trait, especially when their HbA1c value is significantly elevated. This is especially evident when considering the 2 highest HbA1c values in our study (about 15% and 20%). These samples gave markedly improved accuracy when analyzed by the 2nd generation compared to the 1st generation assays.

Several reasons have been offered to explain why higher HbA1c values are observed in HbS and HbC traits by immunoassay compared to HPLC assay. Perhaps HPLC underestimates the true HbA1c values because it expresses HbA1c as a percent of total HbA, whereas immunoassay methods express results as a percent of total Hb. Another explanation is that perhaps immunoassays overestimate true HbA1c values because the amino acid substitutions in HbS and HbC are close to the glycated N-terminus. These are the first 4-10 β-chain amino acids recognized by the immunoassay’s monoclonal antibody [19]. The HbC1c and HbS1c glycated variants, due to altered structure and binding capacity, may have higher affinity for the monoclonal Ab than does HbA1c [14,20]. This has been suggested as the main disadvantage of the Roche “Unimate” 1st generation assay [9]. If monoclonal antibodies can be produced to HbS1c or HbC1c, they could possibly be applied to improve gHb immunoassays.
A previous study suggested testing samples for Hb variants when HbA1c immunoassay values are >15% [21]. With substantially improved immunoassay performance, as observed in our study, such recommendations may no longer be necessary. Further studies should be performed to delineate the performance of the “Tina-quant” immunoassay in samples with other Hb variants, including homozygous conditions. Such studies could provide further insight into immunoassay HbA1c deviations in patients with specific hemoglobinopathies. Additionally, long-term clinical evaluations and outcomes should be assessed based on glycemic control management in diabetic populations with various Hb variants. Such clinical documentation could lead to decreased morbidity and mortality associated with diabetes mellitus.

Acknowledgements

The authors are grateful for support and assistance of Mr. Max Madarressi and the Chemistry staff at Walter Reed Army Medical Center and support and assistance of Mr. Felix Duelm and the Chemistry staff at Brook Army Medical Center.

References