Effects of Azotemia on Results of the Boronate–Agarose Affinity and Ion-Exchange Methods for Glycated Hemoglobin

Mitchell G. Scott, Joseph W. Hoffmann, Victor N. Meltzer, Barry A. Siegfried, and Kwok-Ming Chan

We evaluated the effect of azotemia on results for glycated hemoglobin as measured by a boronate–agarose affinity method and an ion-exchange chromatographic procedure with saline preincubation and found a good correlation. However, values for glycated hemoglobin in samples from nondiabetic patients with various degrees of azotemia were consistently higher with the ion-exchange column procedure (mean, 6.5%) than with the boronate affinity method (mean, 6.2%). The latter method may thus be preferred for monitoring glycated hemoglobin in diabetic patients with impaired renal function.

Measurement of glycated hemoglobin is useful in evaluating long-term control of blood glucose (1–3) and there are numerous methods for doing so: cation-exchange (4), liquid chromatography (5), agarose gel electrophoresis (6), calorimetric and spectrophotometric methods (7, 8), isoelectric focusing (9), immunoassays (10), and a boronate–agarose affinity-column method (11). Each of these methods is subject to various interferences (8).

The method commonly used in clinical laboratories is based on retention of glycated hemoglobin, particularly HbA1, on a column of cation-exchange resin (4). With this procedure, values reportedly are higher than expected for samples from uremic patients (12–15). This has been attributed in part to increased carboxymethylated hemoglobin in these patients (14, 15).

The recently described boronate–agarose affinity chromatography method appears well-suited for the clinical laboratory, and it is not affected by the presence of hemoglobin variants (16) and labile glycated hemoglobin (16, 17). Moreover, values for glycated hemoglobin obtained with this method correlate with other indicators of plasma glucose control in uremic patients (18). Because of the high incidence of progressive renal failure in diabetic patients, we chose to assess the effect of azotemia on results by this method and to determine whether it provides meaningful values for glycated hemoglobin when renal function is impaired.

Materials and Methods

Patients. We analyzed plasma samples from 23 patients without impaired renal function (nonazotemic, serum urea nitrogen <300 mg/L, and creatinine <15 mg/L). These were from diabetics or suspected diabetics for whom glycated hemoglobin evaluation had been requested by physicians. Samples from 22 patients with various degrees of azotemia not requiring dialysis were also selected. They had a mean serum urea nitrogen concentration of >460 mg/L, and a mean serum creatinine of >18 mg/L. All but two of these azotemic patients were nondiabetic, with a blood glucose concentration of <1.40 g/L after overnight fasting. The two diabetic patients had plasma-glucose values >4.0 g/L at the time their glycated hemoglobin was quantified.

Blood urea nitrogen, creatinine, and glucose. Serum urea nitrogen was measured in either the Astra 8 (Beckman Instruments, Fullerton, CA 92634) or the IL 508 (Instrumentation Laboratory, Lexington, MA 02173). Creatinine was determined in either the Astra 8 or the Creatinine Analyzer II (Beckman Instrument, Inc., Fullerton, CA 92634). Plasma glucose was determined in the IL 508.

Glycated hemoglobin. Specimens were collected in tubes containing dipotassium EDTA as anticoagulant and glycated hemoglobin was determined, within 24 h, by an ion-exchange method ("Fast Hb"; Isolab Inc., Akron, OH 44321) and a boronate–agarose affinity method (Glyc-Affin System; Isolab Inc.).

The ion-exchange procedure was performed at ambient temperature, the sample being hemolysate prepared from erythrocytes that had been preincubated for 4 h at 37 °C in isotonic saline to remove labile glycated hemoglobin (19). The results were corrected for variations in ambient temperature according to the manufacturer’s protocol.

Results

Nonazotemic Patients

Values obtained with the ion-exchange method correlated well (r = 0.95) with those obtained with the boronate affinity method for 23 samples from patients with normal renal function (Figure 1A and Table 1). The mean values for the two procedures were 8.6% (range 5.6–13.5%) and 8.5% (range 5.5–14.1%), respectively. A paired t-test showed these means were not significantly different (p > 0.1). The linear regression equation relating the boronate affinity (y) and ion-exchange (x) procedures was: y = 1.065x − 0.672. In addition, both methods exhibited good within-run precision. The CVs for the ion-exchange and boronate affinity methods

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1 Division of Laboratory Medicine, Departments of Pathology and Medicine, Washington University School of Medicine, St. Louis, MO 63110.
2 Chronicl American Kidney Center, Washington University School of Medicine, St. Louis, MO 63110.
3 Department of Pathology and Laboratory Medicine and Department of Medicine, The Jewish Hospital of St. Louis, 216 South Kingshighway, St. Louis, MO 63110.
4 To whom correspondence should be addressed.

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![Fig. 1. Values for glycated hemoglobin Nonazotemic (A) and azotemic (B) patients](image-url)
were ~ 5% and ~ 8%, respectively, for samples with glycated hemoglobin between 5% and 16% of the total hemoglobin.

Azotemic Patients

By comparison, Figure 1B and Table 1 also show the correlation for patients with azotemia. The correlation coefficient was 0.945, and here the linear regression equation was \( y = 0.804x - 0.727 \). Moreover, the ion-exchange method gave consistently higher values. Significantly different (\( p = 0.001 \)) mean values were obtained by the ion-exchange and boronate affinity methods: 8.5% (range 4.0–16.0%) and 6.2% (range 3.6–13.2%), respectively.

Of the 22 specimens assayed, eight (37%) had values above the normal range for the ion-exchange method but within the normal range for the affinity method. These eight patients had normal values for fasting glycemia and showed no clinical signs or symptoms of diabetes mellitus. The mean and standard deviation for glycated hemoglobin for the 20 patients with a blood glucose after overnight fasting of less than 1400 mg/L were 8.0 ± 1.2% and 5.7 ± 0.8% for the ion-exchange and boronate affinity methods, respectively.

**Discussion**

Renal failure is a common complication of diabetes, so techniques that cannot distinguish glycated and carboxamylated hemoglobins may give erroneously high values for the former and compromise the monitoring of glucose control. Whereas values by the ion-exchange and boronate affinity methods correlated well and gave comparable results with samples from patients without azotemia, our patients with various degrees of azotemia gave consistently higher values for glycated hemoglobin by the ion-exchange method than by the affinity method, an effect that persisted even after the erythrocytes were preincubated with isotonic saline to remove the labile glycated hemoglobin (unpublished observation). To assess which method gives results that correlate better with clinical status, we evaluated the two methods, using samples from azotemic but nondiabetic patients. These patients had plasma glucose concentrations of <140 g/L and no history of diabetes mellitus. Values by the boronate affinity procedure were consistently lower than those by the ion-exchange procedure or the reference interval (5.5–8.4%) listed for the affinity procedure by the manufacturer. The amount of nonenzymatically glycated hemoglobin depends on the life span of erythrocytes, which is shortened in patients with azotemia (20). Thus it is unsurprising that patients with impaired renal function could have a lower than expected glycated hemoglobin concentration. Furthermore, glycation of hemoglobin in patients with renal dysfunction can be further impeded by potential competition by carboxamylation (21). This could also result in a lower value in such patients.

The recently described boronate affinity method compares well with the ion-exchange method in speed and ease of performance. Others (16, 17) have found it superior to the ion-exchange method in terms of lack of interference by labile glycated hemoglobin and hemoglobin variants. In addition, our data indicate that the increases in glycated hemoglobin observed in nondiabetic, azotemic patients with the ion-exchange method are not seen with the affinity-chromatography procedure. Our findings suggest that the affinity procedure may be more appropriate for monitoring glycated hemoglobin in patients with impaired renal function and can potentially be the preferred method for routine clinical determinations.

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**References**


Liquid-Chromatographic Determination of Indomethacin in Blood from Newborns with Patent Ductus Arteriosus

Ching-Nan Ou¹,² and Vicki L. Frawley¹

This rapid, accurate "high-performance" liquid-chromatographic procedure is intended for measuring indomethacin in serum from neonates who are receiving indomethacin for symptomatic patent ductus arteriosus. Indomethacin and an internal standard (flufenamic acid) are extracted from serum or plasma with chloroform or diethyl ether at pH 5.0. For the chromatography we used a Waters' Radial Compression Separation System (Radial-NOVA PAK C₁₈ reversed-phase column) and a mobile phase of methanol/sodium acetate buffer (10 mmol/L, pH 3.6), 70/30 by vol. The column effluent is monitored at 254 nm. Both indomethacin and flufenamic acid are eluted within 7 min. Indomethacin can be detected in concentrations as low as 50 μg/L, in 100-μL samples. Response varies linearly with indomethacin concentration to at least 2 mg/L. Analytical recovery is 75%; relative recovery is 100%. Precision is excellent. Using this method, we were able to improve the success rate for pharmacological management of symptomatic patent ductus arteriosus, especially in neonates with fast clearance rates for the drug.

Additional Keyphrases: drug assay · neonates · patent ductus arteriosus

In patent ductus arteriosus (PDA), a congenital heart defect, a small duct between the aorta and the pulmonary artery, which normally closes soon after birth, instead remains open. Symptomatic patent ductus arteriosus is frequently observed in premature infants and can present life-threatening complications. Management of symptomatic PDA included digitalization, diuretics, and eventual surgical ligation until 1976, when Friedman et al. (1) and Heymann et al. (2) reported indomethacin to be effective in the closure of PDA in premature infants in the first postnatal month. Since then, some studies have supported the original observations and others have reported less-impressive responses to indomethacin administration. To identify factors affecting the efficacy of indomethacin in closing the ductus, Brash et al. (3) studied the pharmacokinetics of intravenous indomethacin in 35 premature infants with PDA. They concluded that successful use of indomethacin was associated with a concentration of the drug exceeding 250 μg per liter of plasma 24 h after its infusion. However, they also found a 20-fold variation in these values. Such variation and the relation between concentrations of the drug in plasma and ductus constriction make important the rapid measurement of indomethacin during the 6 to 24 h after the drug is first administered, so that the second dose can be correctly scheduled.

The various methods for determination of indomethacin include spectrofluorometry (4, 5), gas–liquid chromatography (6, 7), radioimmunoassay (RIA) (8, 9), and "high-performance" liquid chromatography (HPLC) (10–16). Spectrofluorimetry is nonspecific, time-consuming, and requires a large volume of sample. Gas–liquid chromatography is more specific but is time-consuming and requires derivatization. RIA, although highly sensitive, is impractical for urgent determinations. Several reported HPLC methods either lack the necessary sensitivity or are subject to endogenous interference. Recently, Mehta and Calvert (17) reported an improved HPLC procedure for monitoring indomethacin in neonates, involving a 10-μm Radial-PAK C₁₈ reversed-phase column. However, this method, a modification of the Skellern and Salole procedure (10), is subject to endogenous interferences, especially for plasma samples.

In 1981, we developed a liquid-chromatographic method for rapidly determining indomethacin in neonates. We have now tested the method over a two-year period and modified it to improve its accuracy and precision. Here, we report in detail this improved procedure.

Materials and Methods

Apparatus. We used a Model 110A HPLC pump (Beckman Instruments, Inc., Berkeley, CA 92634) equipped with a Model 7125 injector (Rheodyne, Inc., Cotati, CA 94928), a Model 440 dual-beam ultraviolet-visible absorbance detector (Waters Associates, Milford, MA 01757), and a radially


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