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SYMPOSIUM

Use of Serial Patient Hemoglobin A1c Differences to Determine Long-Term Imprecision of Immunoassay and High-Performance Liquid Chromatography Analyzers

David V. Tran, B.Sc.,[1] Andrew W. Lyon, Ph.D.,[2] Trefor N. Higgins, M.S.,[3] James C. Wesenberg, Ph.D.,[4] Lisa Vandergouwe, B.Sc.,[5] Carmen L. Wiley, Ph.D.,[6,7] and George S. Cembrowski M.D., Ph.D.[1]

Abstract

Background:

The quality of the HbA1c assay is inversely proportional to the variation of the assay. Most published measures of HbA1c variation are limited by the data collection period, the statistical treatment of outliers, and even the noncommutability of the products used to generate the variation measurements. We have used an alternate approach to derive HbA1c variation, using serial patient data.

Methods:

HbA1c measurements of outpatient blood sample pairs drawn within 30 days of each other were made on three different immunoassay systems: the Roche INTEGRA® 700, the Roche INTEGRA® 400, and the Dade Dimension® RxL; and two high-performance liquid chromatography assays: the Tosoh G7 and the Tosoh 2.2+. The standard deviation of duplicates was calculated for the following time intervals: 1 to 3 days, 4 to 6 days, 7 to 9 days, . . . , 28 to 30 days. These intra-individual variations were then plotted; extrapolation to time zero yields the long term total random error which consists of both analytic and pre-analytic error. Data collection periods were usually 2 years.

Results:

At the mean HbA1cs of 7.08%, 7.14%, 7.20%, 6.96%, and 7.51% for populations tested on the Roche INTEGRA 700, Roche INTEGRA 400, Dade Dimension RxL, Tosoh 2.2+, and Tosoh G7, respectively, the total analytic imprecisions (coefficient of variation) were 2.56%, 2.29%, 2.25%, 1.66%, and 1.14%, respectively.

Conclusion:

Assessment of the HbA1c long term total imprecisions shows that while the three immunoassay systems are acceptable, the Tosoh HbA1c analyzers demonstrate superior analytic performance.

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Author Affiliations: [1]University of Alberta, Edmonton, Alberta, Canada; [2]University of Calgary and Calgary Laboratory Services, Calgary, Alberta, Canada; [3]DynaLIFEDX, Edmonton, Alberta, Canada; [4]David Thompson Health Region, Red Deer, Alberta, Canada; [5]Chinook Health Region, Lethbridge, Alberta, Canada; [6]Marshfield Laboratory, Marshfield, Wisconsin; and [7]currently with Providence Sacred Heart Medical Center, Spokane, Washington

Abbreviations: (CAP) College of American Pathologists , (CV) coefficient of variation, (HbA1c) Hemoglobin A1c, (HPLC) high-performance liquid chromatography, (NACB) National Academy of Clinical Biochemistry, (NGSP) National Glycohemoglobin Standardization Program, (SDD) standard deviation of duplicates

Keywords: hemoglobin A1c, long-term analytic variation

Corresponding Author: George Cembrowski, M.D., Ph.D., 4B1.24, 8440 112 Street, Edmonton AB T6G2B7, Canada; email address cembr001@cha.ab.ca

Introduction

The usefulness of measuring HbA1c to assess glycemic control in patients with diabetes mellitus is now well established. Following the publication of the Diabetes Control and Complication Trial[1] and the United Kingdom Prevention of Diabetes Study,[2] there were attempts on many fronts to improve the quality of HbA1c testing. Specifically, the National Glycohemoglobin Standardization (NGSP) program has achieved remarkable success in decreasing the imprecision in HbA1c testing.[3] This improvement is demonstrated in North American laboratory performance of glycohemoglobin unknowns sent out by the College of American Pathologists (CAP) proficiency testing program.

Patient and mathematical specifications have been used to derive imprecision goals for HbA1c analysis. It is remarkable that most of these approaches have concluded that an imprecision goal between 2 to 3% is desirable for HbA1c. The National Academy of Clinical Biochemistry (NACB) recommends a target imprecision of 3% in both the published 2002[4] and the draft 2007 guidelines. Traditional quality control methods have been used to assess the imprecision of HbA1c methods. The NACB guidelines recommend the performance of two levels of control with each HbA1c run to assess within-laboratory imprecision. The CAP glycohemoglobin proficiency program provides data on the inter-laboratory imprecision of various HbA1c methods.

Estimates of imprecision are usually based on the repeated analysis of quality control specimens. These estimates are dependent on the matrix of the control specimen which may differ significantly from actual patient specimens.[5,6] Furthermore, these quality control specimens are treated very differently than patient specimens. Too often, outlying points may be visually eliminated without testing for statistical outliers.

We have developed a unique approach to determining the imprecision of HbA1c methods. Summaries of intra-individual HbA1c variations are plotted against the time between sampling. Extrapolation to zero time yields a combination of biologic and analytic variation. Since HbA1c is slow-forming and its biological variation is low[7] and is partly time-dependent, we have assumed that the majority of this variation at time zero is due to analytic imprecision. Previous evaluations have mainly focused on high-performance liquid chromatography (HPLC) HbA1c methods, including comparing an HPLC and immunoassay method within a university laboratory[8] and evaluating the performance of three identical HPLC systems in a large referral laboratory.[9]

Here, we use our approach to analyze HbA1c data from three Alberta laboratories that use different immunoassay methodologies, and Alberta and Wisconsin laboratories that use HPLC. The immunoassays are represented by (1) a laboratory that serves a large metropolitan area and multiple teaching hospitals, (2) a mid-sized hospital laboratory located in a community of 100,000 people, and (3) a rural hospital serving a large aboriginal population. One HPLC is used by a regional south Alberta laboratory that provides health care to 150,000 people, half of whom are engaged in the service industry.

The other HPLC is used in a Wisconsin laboratory that supports a group practice of 792 physicians in 80 medical specialties and subspecialties located in over 40 centers throughout northern, central, and western Wisconsin. This regional reference laboratory provides testing to 115,000 people, most of whom are engaged in the agricultural industry. Marshfield Clinic's Diabetes Guidelines are adapted from the Institute for Clinical Systems Improvement guidelines (*http://www.icsi.org/guidelines_and_more/*) and they participate in the Medicare Care Management Performance Demonstration Project.

Methods

Patient glycohemoglobin measurements were performed on one of four different analyzers in Alberta, Canada: the Roche INTEGRA® 700 (Roche Diagnostics Canada Ltd, Laval, Canada) in Calgary, the Roche INTEGRA® 400 in Wetaskiwin, the Tosoh G7 (Tosoh, Grove City, OH) in Lethbridge, and the Dade Dimension® RxL in Red Deer (Siemens, Tarrytown, NY). The Tosoh 2.2+ is used by Marshfield Clinical Laboratories in Marshfield, Wisconsin. All instruments were run according to the manufacturers' standard operating procedures.

Approximately 2 years of patient data were available from each site; all data were extracted from the long-term data repositories of individual laboratory information systems: 317,100 Calgary HbA1cs were collected between March 1, 2005 and March 31, 2007; Red Deer (36,400 HbA1cs) and Wetaskiwin (12,900 HbA1cs) between January 1, 2006 and December 31, 2007; and Lethbridge (50,900 HbA1cs) between April 1, 2006

and March 31, 2008. There were 43,114 HbA1cs from Marshfield collected between October 2, 2006 and September 30, 2008.

Data Analysis

As some of the individual HbA1c results were expected to be significant outliers that could greatly confound our calculations, frequency histograms of each set of test results were inspected to visually determine appropriate exclusion (truncation) limits. Statistical percentile ranking of the histograms supported setting HbA1c limits of 4 to 12%; at least 98.6% of all results fell within these limits.

For each site, Microsoft Excel (Microsoft Corporation, Seattle, WA) was used to select all intra-patient pairs of HbA1c samples within 4 to 12% and sampled within 30 days of each other. The paired patient results were grouped into the following 3 day intervals between samplings: 0 to 3 days, 4 to 6 days, 7 to 9 days, . . ., 28 to 30 days (yielding 10 intervals). The average variations in these groups of paired HbA1c were calculated from the standard deviation of duplicates formula (SDD)[10]:

$$SDD = \sqrt{(\Sigma(x_{i1} - x_{i2})^2/2n)}$$

Finally, the SDD values were graphed against the average time interval in which they were divided, and linear regression was used to determine the y-intercept. Extrapolation to time zero yields the long-term total random error which consists of both analytic and pre-analytic error. We assumed that the pre-analytic error was near zero due to the inherent stability of the HbA1c moiety and the universal usage of bar-coded specimens and computer-interfaced analyzers. The long-term coefficient of variation (CV) for each site was calculated by dividing the y-intercept value by the HbA1c population mean.

Results

Figure 1 presents the regression plots of grouped SDDs for each laboratory; intercept error is presented as the percentage of the intercept (i.e., intercept error/intercept × 100%).. The three immunoassay regression lines are almost superimposable, having almost the same slopes and y- intercepts. The two HPLC lines are close to each other and almost parallel to the immunoassay lines. As the correlation coefficients for the INTEGRA 400 and the Tosoh G7 were less than 0.90, an alternate regression technique,[11] Passing-Bablock,[12] was used to derive the slope and intercept. For the INTEGRA 400, the regression statistics were unchanged; for the Tosoh G7, the slope

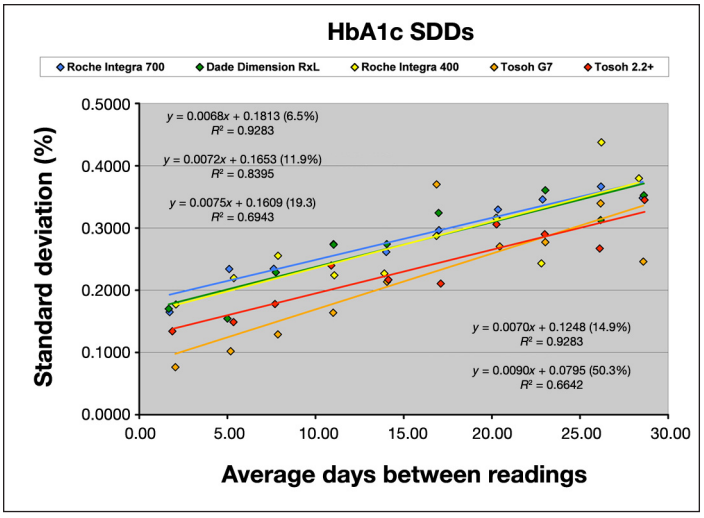

Figure 1. Standard deviation of duplicates (SDDs), in units of % HbA1c for each time interval representing intra-individual variations, are regressed over time. Intercept errors are presented as percentages in parentheses. Extrapolation to time zero yields the long term total random error which consists of both analytic and pre-analytic error.

increased to 0.011 from 0.0090 and the intercept dropped to 0.050 (95% confidence interval 0.028 to 0.082) from 0.0795.

Table 1 summarizes the number of HbA1c pairs in each time interval up to 30 days for each instrument. **Table 2** provides a statistical summary of the superset of HbA1cs ordered over 2 years in each region, as well as of the subset of HbA1cs used to calculate the imprecisions.

Division of each y-intercept in **Figure 1** by the respective population mean HbA1c (7.08%, 7.14%, 7.20%, 7.51%, and 6.96% for populations tested on the INTEGRA 700, INTEGRA 400, RxL, 2.2+, and G7, respectively), yielded total analytic imprecisions (CV) of 2.56%, 2.29%, 2.25%, 1.66% and 1.14%, respectively. Use of the Passing-Bablock intercept for the G7 resulted in a lowering of the CV from 1.14% to 0.72%.

Discussion

Using our approach, the total analytic variation is attributed to the variation in multiple reagent lots, multiple calibrators, and multiple calibrations during the study period, as well as long- and short-term environmental differences, including variations in ambient temperature. Our analysis takes into consideration the variation observed in specimens that are separated over periods as long as 30 days; these periods overlap for a 24 month interval. The variation of the patient duplicates is thus a measure of the average variation over this extended period.

Table 1.
HbA1c Pairs Within 30 Days for Each Instrument.

Interval when pair was taken, days	Roche INTEGRA 700	Dade Dimension RxL	Roche INTEGRA 400	Tosoh G7	Tosoh 2.2+
1-3	1096	98	25	41	47
4-6	881	83	25	38	32
7-9	1460	168	53	81	66
10-12	871	83	32	30	46
13-15	1723	179	50	60	63
16-18	957	106	35	53	49
19-21	1583	191	42	76	65
22-24	1186	120	41	62	57
25-27	1467	142	61	66	75
28-30	3882	347	219	119	98
Total	15,106	1517	583	626	598

Table 2.
Means of HbA1c populations and paired subsets.

	Roche INTEGRA 700	Dade Dimension RxL	Roche INTEGRA 400	Tosoh G7	Tosoh 2.2+
Location	Calgary	Red Deer	Wetaskiwin	Lethbridge	Wisconsin
Dataset, *n*	317,100	36,400	12,900	50,900	43,114
Mean HbA1c	6.53	6.84	6.75	6.59	6.87
Dataset generating pairs within 30 days, *n*	25,682	2683	1026	1162	1309
Mean HbA1c	7.08	7.20	7.14	6.96	7.51
Fraction of total tests repeated within 30 days, %	8.10	7.37	7.95	2.28	3.04

The long term standard deviations of the Tosoh analyzers are roughly one-half to three-fourths of the immunoassay systems and are amazingly low. This superior performance of the Tosoh HPLC system is noted on the CAP Glycohemoglobin survey program where, over the past 2 years, the analytical performance, expressed as a CV, has been consistently lower than any other method for calculating HbA1c values. Further review of the CAP Proficiency survey shows that the Tosoh HPLC represents approximately 15% of HbA1c methods whereas the three different immunoassay systems that we evaluated account for approximately 30% of HbA1c analyzers. The rationale for using immunoassay methods for HbA1c analysis is based on ease of use and the capability of automation rather than any analytical superiority either in precision or the ability to detect hemoglobin variants. All methods meet the ideal target imprecision of the NACB recommendations and are well within the recommended imprecision of 5%.

In an earlier report of analytical imprecision of HbA1c methods based on methods in the Edmonton Alberta region, the Bio-Rad VARIANT II HPLC method had better precision (around 3%) than immunoassay systems (5%).[8] This work was performed on patient data generated 4 years ago, and manufacturers of HbA1c methods, both immunoassay- and HPLC-based, have made modifications to their methods to improve precision. This desire to improve the precision stems from market driven competition and the increasingly stringent demands of the NGSP.

The issue of bias of HbA1c methods has become of vital significance[13] with the introduction of target therapeutic goals for the treatment of patient with diabetes (American Diabetes Association recommend a treatment goal HbA1c of 7%). A particular HbA1c method may have superb precision but produce results that are significantly different than other methods leading the clinician to

over- or under-medicate. The method described in this paper, using repeat patient data, was designed to evaluate method imprecision and cannot be used to accurately evaluate bias. To evaluate bias, one requires a comparison of HbA1c results produced on identical samples and a comparison against an accepted assigned value, such as the CAP glycohemoglobin proficiency program using NGSP assigned values. The combination of the use of HbA1c to screen for diabetes and patient acuity may vary between the different locations, making it impossible to establish method bias from the mean and median HbA1c values.

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