Standardization of Hemoglobin A1c

To the Editor:
Kobold et al. (1) describe candidate reference methods for hemoglobin A1c (HbA1c) that are based on the identification of hexapeptides from the N-terminus of the β-chains by both a mass spectrometry technique and capillary electrophoresis. Although they are to be congratulated on their efforts in this regard, I wonder whether the (IFCC) definition of HbA1c as hemoglobin modified irreversibly by glucose at one or both N-terminal valines of the β-chains might be a source of difficulty when applied to routine methods. For example, if a patient’s specimen contains, as seems probable, a mixture of modified hemoglobin species such that 10% have both N-terminal valines glycated (β2-G2) and 5% have only one valine glycated (β2-G), the candidate reference methods will determine a HbA1c proportion of 12.5% because this represents the proportion of N-terminal valines that are glycated. It appears unlikely that any routine method will duplicate this result other than by chance. Rather, measurement of intact tetrameric hemoglobin will produce a result of either 10% if only β2-G2 is recognized as HbA1c or 15% if both β2-G2 and β2-G are so recognized. Moreover, it seems possible that these considerations underlie the slope of 0.824 that was found when Mono S chromatography was compared with mass spectrometry [Fig. 8 of (1)]. If β2-G is included in the non-A1c fractions on Mono S, mixtures containing an appropriate proportion of this species, e.g., 10% β2-G2 plus 42% β2-G, will give results of 10% for chromatography, 12.1% for mass spectrometry, and a ratio of 0.826 for the comparison, close to the observed ratio. In consequence, routine methods for HbA1c can only be expected to agree with the proposed reference methods when the specimens that are analyzed are devoid of β2-G.

The authors of the reference cited above respond:

To the Editor:
The letter of Johnson (1) addresses an important question about the structure and behavior of glycated hemoglobins in cation exchange chromatography systems. In the literature, only limited information on this topic is available, which produces misunderstanding and confusion.

The IFCC Working Group on the Standardization of Hb A1c has extended the definition of Goldstein et al. (2), which states that Hb A1c represents Hb A0 irreversibly glycated at one or both N-terminal valines of the β-chains of the tetrameric hemoglobin molecule. This includes hemoglobins that may be additionally glycated at lysine residues. Species glycated only at lysines are not considered to be Hb A1c.

Any N-terminal glycation of β- or α-chains will change the pK of the amino group of valine. These glycated hemoglobins will elute together in the Hb A1c peak on cation exchange chromatography systems. From the in vitro experiments of Weykamp and Penders (3), we know that the major glycation product is singly glycated hemoglobin. To a small extent, the double Schiff base is also formed in increasing amounts with time and glucose concentration, but the double Amadori product Hb A1c cannot be observed. Therefore, tetramers glycated on both N-terminal β-chains seem to represent only a minor fraction.

The two candidate reference methods measure the total amount of glycated β-chains (4), as do most common cation exchange chromatography systems. Recent gel filtration experiments using buffers with pH <6.2 have demonstrated that, under these conditions, hemoglobins are eluted with a molecular weight of ~34 kDa, indicating a dissociation of the tetrameric Hb A1c molecule into α/β-dimers at this pH (5) and A. Finke, U. Kobold, W. Hoelzel, C. Weykamp, K. Miedema, and J-O. Jeppsson, manuscript submitted for publication. The Mono S system, for example, operates at pH 5.7 and thus quantifies dimers.

The correlation between Mono S and the candidate reference methods is still difficult to explain (4). Under the chromatographic conditions used, the response factors for Hb A1c and Hb A0 are not identical at a detection wavelength of 415 nm. Additionally, we know that the Hb A1c peak on Mono S and other cation exchange systems contains α/β-dimers as a complex mixture of mono- and multiple-glycated hemoglobins, together with carboxylated and acetylated species. No cation exchange chromatographic system, therefore, is specific for Hb A1c, and thus does not reflect the true value for Hb A1c.

Most chromatographic as well as immunological methods are operated at a pH where one can expect the existence of monomeric or dimeric hemoglobin peptide chains. Thus, these Hb A1c determinations are independent of the glycation status of the tetrameric Hb A1c molecule as it may exist under physiological conditions.

References
To the Editor:

Recently Bean et al. (1) compared isoelectric focusing/immunoblotting/laser densitometry (IEF/IB/LD), Axis %CDT TIA, and Axis %CDT HPLC for the determination of CDT. This study shows shortcomings that need further discussion.

1. The three methods, as presented in Materials and Methods in that paper, are not semiautomated. The statement that “similar results are obtained on an array of turbidimetric instruments” on page 987 cannot be deduced from the data presented. None of the 10 analyzers mentioned was tested in this study, and appropriate references are not given. Thus, the title does not reflect the content of the paper.

2. On page 983, the authors state that “inclusion of the trisialotransferrin fraction increases the accuracy in the diagnosis of sustained alcohol usage.” However, on page 988, the discussion ends with “whether the inclusion of the trisialotransferrins results in improved efficacy of the test awaits further analysis.”

3. Predictive values, diagnostic sensitivities, and specificities strongly depend on the definition of false positives and negatives and patients’ sex and liver function (2). However, no information is given on how daily alcohol consumption for groups 1–4 was verified. Furthermore, groups 2–4 combine men and women (with and without alcohol-induced liver disease). Although gender-specific upper reference limits of CDT are described in detail and generally accepted (2), the authors establish gender-nonspecific cutoffs without giving any reasons for this procedure.

4. The study illustrates the need for a unified definition of CDT. On page 983, the IEF/IB/LD is said to summarize asialo-, mono-, and distialotransferrins as CDT; on pages 984, 987, and 988, only asialo- and distialotransferrin. %CDT TIA analyzes asialo-, mono-, di-, and 50% of trisialotransferrin; %CDT HPLC mono-, di-, and 50% of trisialotransferrin, but not asialotransferrin (pages 984–985). IEF/IB/LD measures partially iron-loaded, %CDT TIA, and %CDT HPLC iron-saturated isotransferrins. Thus, CDT values obtained by three methods, measuring different analytes as CDT, are tested for equality in this study. How the inclusion of 50% trisialotransferrin in CDT determination by %CDT TIA and %CDT HPLC was verified and tested for reproducibility is not described or cited. On page 986, the authors discuss the “separation of CDT from fully sialylated transferrin isoforms” without defining the latter term, although asialo- to octasialotransferrins have been described. Thus it remains unclear which transferrins are meant.

5. Cofocusing or coelution of tetrasialotransferrin with CDT causes false positives (3, 4). Therefore, tetrasialotransferrin must and not “should” be “avoided in the eluate” (page 984).

6. A similar correlation between IEF/IB/LD and %CDT TIA and between IEF/IB/LD and %CDT HPLC was obtained not “because all three tests measure relative CDT . . . rather than absolute CDT . . . ” as concluded by the authors, but because the %CDT TIA minicolumn performance was “calibrated until the correlation between HPLC and TIA methods was at maximum”.

7. Lanes a and b and c and d have been exchanged in the legend of Fig. 1. In interpreting Fig. 1, monosialotransferrin is ignored, although its bands are clearly visible.

8. In contrast to present knowledge (2, 5), genetic transferrin D variants did not cause false positives in this study. This finding should have been discussed by the authors.

9. Whether for %CDT TIA “each specimen requires two measurements” or only one because “its precision is low enough to allow for single determination of CDT. . . .” (page 987) remains unclear.

10. Superficial errors, e.g., “Grøbæk” instead of “Großbach”, “Sundrehagen” instead of “Sundrøningen” (one of the co-authors) further weaken the merit of the study.

Because of these shortcomings, the study does not really contribute to a better understanding of the diagnostic performance of CDT as a marker of chronic alcohol abuse.

References


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