Comparing Multiple Measures of Glycemia: How to Transition from Biomarker to Diagnostic Test?

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The underlying pathophysiology of diabetes varies, but all patients share a common a metabolic derangement of carbohydrate metabolism, which causes hyperglycemia. Many patients with diabetes develop debilitating complications, ranging from retinopathy and nephropathy, to myocardial infarction and stroke. The accumulated evidence reveals that reducing an increased glucose concentration, as documented by a lower hemoglobin A1c (Hb A1c)4 concentration, decreases complications (1, 2). Hb A1c is extensively used to monitor glycemic control and to adjust therapy, and it has recently been accepted as a criterion for the diagnosis of diabetes (3).

Hb A1c reflects long-term glycemia, because glucose attaches irreversibly (the process is termed glycation) to hemoglobin in erythrocytes. Hb A1c can be modified independently of glycemia, however, by conditions (e.g., anemia or renal failure) that alter the mean age of erythrocytes by changing either their production or their rate of disappearance. Recent observations have revealed that the life span of erythrocytes in healthy individuals who have normal blood counts and indices can vary sufficiently from the commonly taught 120 days to cause clinically relevant differences in Hb A1c concentrations (4). These limitations have led to investigations of an expanding group of alternative markers of glycemia.

The best studied of these analytes is fructosamine, which is the generic name for plasma protein ketoamines. Because albumin is the most abundant protein in serum, fructosamine is predominantly a measure of glycated albumin. The covalent attachment of glucose to albumin forms glycated albumin, which can also be assayed directly (5). The half-life of albumin in the blood is 14–20 days, so both fructosamine and glycated albumin indicate the mean blood glucose concentration over the preceding 2 weeks. The fructosamine assay was developed in 1983 (6), but it was modified—and improved—in 1990 by the addition of uricase, a nonionic detergent, and polylysine calibrators (7). Automated assays of glycated albumin have been available for approximately 10 years. Fructosamine and glycated albumin are extracellular and have several useful attributes, including independence from both erythrocyte life span and glucose transport across membranes.

The assays are rapid, technically easy, and inexpensive; however, changes in protein concentration and half-life affect fructosamine, and whether the results need to be corrected for albumin concentration remains controversial. Glycated albumin is also altered by conditions other than glycemia, including the nephrotic syndrome, thyroid dysfunction, hepatic cirrhosis, smoking, hyperuricemia, and hypertriglyceridemia (5).

Another indicator of glycemia is 1,5-anhydroglucitol (1,5-AG), a 6-carbon monosaccharide that is not metabolized (8). Because glucose in the urine competes for reabsorption of 1,5-AG by the kidneys, blood glucose concentrations that exceed the renal threshold [usually approximately 180 mg/dL (10 mmol/L)] reduce the circulating 1,5-AG. The clinical value of 1,5-AG as a marker of short-term glycemia, especially the postprandial glucose concentration, is limited by other factors that modify 1,5-AG, including diet, sex, and renal impairment (8). Although several assays for these alternative markers are commercially available, these analytes all suffer from a major deficiency: clinical studies have been limited, and there is a paucity of rigorous analysis in the literature. For example, the number of PubMed hits in humans for fructosamine, glycated albumin, and 1,5-AG is 3.75%, 2.3%, and 0.35%, respectively, of that for Hb A1c.

In this issue of Clinical Chemistry, Juraschek et al. describe their study of the relationships of fructosamine, glycated albumin, and 1,5-AG to Hb A1c and the fasting glucose concentration in a community-based cohort (9). The authors report that nonlinear equations best fit the relationship of fructosamine and glycated albumin with both Hb A1c and fasting glucose, albeit with considerable scatter. A careful inspection of Table 2 in their report, however, reveals 2 important caveats. The first is that a major proportion of the correlation in the population as a whole depends on the
population subset with a history of diagnosed diabetes, which constitutes only 20% of the study participants (the relationships by any equation are very limited when individuals with diabetes are excluded). The second observation is that for the individuals with diabetes, the improvement in $r^2$ achieved by going from a linear fit to a nonlinear fit is modest. At diabetes diagnostic thresholds based on Hb A1c and fasting glucose, Juraschek et al. observed wide confidence intervals for values derived from the best-fit equations for the alternative markers. They appropriately limit their interpretation to conclude that fructosamine and glycated albumin are useful adjuncts to Hb A1c and glucose, and refrain from extrapolating their findings to propose values for glycated albumin or fructosamine for diagnosing diabetes.

The study generates an important question: Can diagnostic criteria for diabetes and related dysglycemic states be established interchangeably among these analytes to permit the use of an alternative marker for situations in which glucose and Hb A1c are unreliable? The insights gleaned from applications of continuous glucose monitoring are valuable in considering this issue. Such monitoring provides very frequent measurements of blood glucose over 2 to 3 days, thereby permitting a reasonable assessment of mean glucose concentrations. The data allow determination of how precisely Hb A1c reflects integrated glycemia. Although Hb A1c and mean blood glucose are highly correlated, even in the most carefully obtained data set published, considerable heterogeneity or scatter in the relationship between the 2 parameters is observed (10, 11). Some experts attribute this variation to limitations in assay accuracy, whereas others ascribe it to interindividual differences in the physiological determinants that underlie each measure. It is likely that both factors contribute. Particular concern regarding differences by race has been expressed (12–15).

Diabetes is a consequence of both genetic and environmental factors and has a variable course. Because false-positive and false-negative determinations of diabetes have considerable implications for individuals and society, diagnostic criteria have been established through compromise by selecting defined cutpoints along a continuous variable. What constitutes a “gold standard” for diagnosis thus represents a compromise, balancing benefits with risks. Historically, glucose measurement (in the fasting or postprandial state) was used as the sole diagnostic criterion. The convenience and advantages that Hb A1c analysis offers over blood glucose measurement (16) led to the adoption of Hb A1c as an optional diagnostic test for diabetes (3). A plethora of reports have recognized the limited sensitivity of Hb A1c at the threshold (Hb A1c, 6.5%), which was selected to have a high specificity and adequate sensitivity to detect diabetes defined by the glucose criteria and to identify diabetic retinopathy. Unfortunately, some patients are classified as having diabetes or prediabetes by one measure (glucose) but not by the other (Hb A1c), and vice versa (15, 17). The heterogeneity in the relationship between the mean blood glucose concentration and Hb A1c produces a fairly wide distribution of points around a line when the 2 measures are expressed graphically. Defining a diagnostic threshold by a sharp cutoff in the middle of such a region results in some people who meet one criterion falling on one side of that cutoff (i.e., meeting the criterion for diabetes) and others falling on the opposite side (i.e., not fulfilling the diagnostic criterion). Therefore, heterogeneity likely contributes to the discrepancies that have been noted between sensitivity and specificity in the translation of glucose-based criteria to Hb A1c-based diagnostic criteria.

The problem would be compounded if glucose and Hb A1c were considered equivalent “gold standards” for selecting thresholds for diagnosis with alternative markers, which would make the next marker(s) more difficult to interpret for diagnosis. Should that standard be a serum marker like glycated albumin, fructosamine, or 1,5-AG? Should it be a physical marker that offers the advantages of being noninvasive and available in a setting accessible to those who do not or cannot access medical care? Examples include assays under development that use fluorescence to measure advanced glycation end products in either the skin or the eye. These issues merit contemplation, but the simple answer is a preference for limiting use of the multiple measures to screening and for remaining as close as possible to a “gold standard” for diagnosis. Perhaps it is time to establish a science of “glycomics.”

The study by Juraschek et al. also illustrates the challenges inherent in the selection of appropriate populations for different steps in the process of extending diagnostic criteria to alternative markers and overcoming confounding factors due to intercurrent disease states. Table 2 of their report demonstrates that although the determinants of glycated albumin and fructosamine are similar, the best-fit equations for the relationships of these 2 markers to Hb A1c do not even take the same form, much less have similar parameter values, for people with diabetes, compared with those without the disease. These data demonstrate how dependent the fit for the entire population is on the distribution of diabetes in the study population. This observation implies that the process for evaluating potential new diagnostic markers for diabetes should comprise 2 parts. The first should use a population with substantial numbers of individuals who are distributed across the entire range of glycemic control. This component of the study would generate a com-
mon equation, which is likely to closely reflect the physiological determinants of the relationship. The second segment of the analysis would assess diagnostic performance in a general population cohort, analogous to the Atherosclerosis Risk in Communities (ARIC) Study used by Juraschek et al. Similar considerations pertain to the ability to distinguish the presence of diabetes from its absence in the context of confounding nephropathy. Accurate discrimination is not possible with a study population that has a limited representation of patients with nephropathy and a narrow range of proteinuria with limited measures of total serum proteins.

Notwithstanding the considerations alluded to above, the study by Juraschek et al. enhances our knowledge of alternative markers of glycemia. It also advances the discussion by drawing attention to key issues that need to be addressed in future research so that these relationships can be dissected out, whether for comprehension of pathophysiology, for assessing the risk of complications, or for diagnosis of diabetes.

References


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