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The quest for the perfect biomarker of long-term glycemia: new studies, new trials and tribulations

Frequent evaluation as well as precise measurement of glycemic control form a critical part of diabetes management because the success of treating diabetic patients depends, to a large extent, on the attainment of target glycemic levels. Currently, glycated hemoglobin (HbA_{1c}) remains the most widely used test for the assessment of glycemic control. HbA_{1c} is formed from the nonenzymatic glycation of hemoglobin in erythrocytes and provides a reliable reflection of average glycemic status in the preceding 2 to 3 months, allowing clinicians to evaluate success of their treatment and also make treatment adjustments based on its measurement. HbA_{1c} has been well correlated with diabetes-related micro- and macrovascular complications in large epidemiological trials [1,2] and can be conveniently measured in the clinics without fasting or any other special preparations. Concerns with inconsistencies of HbA_{1c} assays have been alleviated to a large extent by the widespread standardization of HbA_{1c} by the National Glycohemoglobin Standardization Program to the Diabetes Control and Complications Trial standards. Recently, the American Diabetes Association (ADA) extended the utility of HbA_{1c} by including a cutoff of more than 6.5% as a diagnostic criterion for diabetes mellitus [3]. The ease of performing HbA_{1c}, a test with relatively low day-to-day fluctuations and/or fluctuations due to stress and illnesses, lends further support to using HbA_{1c} as a diagnostic test for diabetes. Besides its role as a measurement of glycemic control and diagnostic tool for diabetes, studies have described HbA_{1c} as a reliable tool for diabetic risk stratification, with higher-than-normal levels being predictive of future development of diabetes [4,5]. The American Diabetes Association has endorsed the HbA_{1c} range of 5.7% to 6.4% as a category of increased risk of diabetes [6]. The associations with higher HbA_{1c} levels in diabetes and risk of micro- and macrovascular complications are well documented, as is the relationship between the risk of retinopathy and HbA_{1c}, which is similar to that with fasting plasma glucose and 2-hour post load oral glucose tolerance test [3]. Moreover, HbA_{1c} has been found to be an independent predictor of cardiovascular events in certain nondiabetic populations [7], further extending its clinical utility.

Glycated hemoglobin has its fair share of limitations. Despite the association with cardiovascular risk, the failure of the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation study, the Action to Control Cardiovascular Risk in Diabetes trial, and the Veterans Affairs Diabetes Trial to demonstrate consistent outcome benefits with intensive control of diabetes that resulted in lower HbA_{1c} levels suggested that the relationship between HbA_{1c} levels and cardiovascular risk is not plain linear or straightforward [8-10]. The proposed "legacy effect" of early glycemic control may not be directly and fully captured by assessment of glycemic control in the short term as is the case by using HbA_{1c}. The limited availability or standardization of the test in the developing world, the relatively higher cost, the inconsistencies of HbA_{1c} in different ethnic groups [11], the effect of chronic renal diseases, anemias, and specific conditions with increased red cell turnovers such as blood transfusions and hemolysis that tend to underestimate HbA_{1c} have limited its practicality in many settings [12,13]. Furthermore, hemoglobinopathies such as sickle cell traits (hemoglobin S) and other abnormal hemoglobin variants such as hemoglobin C and E variants can lead to falsely high or low HbA_{1c} readings depending on the laboratory methodology used [14].

Other non-HbA_{1c} biomarkers of glycemic control have been described, with each of them exhibiting characteristics complementing the utility of HbA_{1c} in several different ways. Fructosamine is formed from nonenzymatic glycation of protein molecules to form stable ketoamines [15]. Serum concentrations of fructosamine reflect glycemic control in the preceding 2 to 3 weeks. Not only that a reasonable correlation exists between fructosamine and HbA_{1c} [16], one distinct clinical advantage of this test is that fructosamine responds more quickly to changes in blood glucose levels, allowing for early and timely adjustment of diabetic treatment decisions [15]. Moreover, fructosamine is not affected by red cell turnover or hemoglobinopathies, allowing its use in specific clinical conditions that exclude the use of HbA_{1c}.

Another non-HbA_{1c} biomarker, glycated albumin (GA), similar to fructosamine, is dependant on the nonenzymatic glycation of albumin and is used as a marker of glycemic control in the preceding 2 to 3 weeks [17]. Besides sharing many advantages similar to fructosamine, as it is independent of hemoglobin integrity, GA was reported to be a more accurate reflection of glycemic control in diabetic hemodialysis subjects compared with HbA_{1c} [18,19]. However, both fructosamine and GA, being dependent on glycation of protein, will not be a reliable marker in conditions in which

protein metabolism is altered such as in nephrotic syndrome and liver cirrhosis [20,21]. An article in the present issue of *Metabolism* evaluated the influence of fatty acids, which are major endogenous ligands, on albumin glycation and of glycation on albumin conformation and exogenous ligand binding [22]. Findings indicate that fatty acids impede the ability of albumin to undergo Amadori-glucose modification and to induce conformational changes affecting exogenous ligand binding. It also demonstrates that nonenzymatic glycation of albumin induces alterations in structural and functional properties that may have important implications in lipid transport and atherogenesis [23].

1,5-Anhydroglucitol (AG), a naturally occurring dietary polyol, has been proposed as a measurement of short-term glycemic control and glucose variations over 1 to 2 weeks [24]. 1,5-Anhydroglucitol reflects glycemic control via the mechanism that renal reabsorption of 1,5-AG is competitively inhibited by serum glucose. When glucose levels rise, albeit transiently, urinary loss of 1,5-AG increases; and serum levels fall and serve as a marker of postprandial glucose excursion. A lower level reflects a larger postprandial glucose excursion and vice versa. Postprandial glucose excursion may be an independent risk factor for development of macrovascular complications [25,26], and postprandial hyperglycemia might be prominent even in patients with optimal HbA_{1c} [27]. Thus, 1,5-AG is attractive in that it measures glucose excursions not directly captured by measurement of HbA_{1c}, and may possibly have a complementary role to HbA_{1c} in the fine tuning of glycemic control in moderately controlled diabetic patients [28]. However, 1,5-AG may not be an accurate marker of glycemic control in subjects with advanced renal failure [29]. It also remains to be seen whether the new class of sodium-glucose cotransporter 2 inhibitors would interfere with the levels and, by extension, predictive value of this test.

The issues of cost and limited availability of these non-HbA_{1c} biomarkers aside, specific deficiencies of these tests as a group do exist. These non-HbA_{1c} markers have not been evaluated definitively and/or comparatively to HbA_{1c} as a lone diagnostic tool for diabetes. Moreover, perhaps the most significant shortfall of these non-HbA_{1c} biomarkers of glycemic control in comparison with HbA_{1c} lies in the relative paucity of data to date demonstrating long term outcome benefits in using these markers in the management of diabetes. Unlike HbA_{1c}, whose outcome benefits with its utilization have been validated in several large trials including the United Kingdom Prospective Diabetes Study and Diabetes Control and Complications Trial, these non-HbA_{1c} biomarkers are still awaiting further long-term outcome studies before they can find their way into the standard armamentarium of diabetes management.

Despite these shortcomings, studies describing associations of diabetic complications with these non-HbA_{1c} biomarkers have shown promise in terms of their clinical utility. Glycated albumin and fructosamine are reportedly positively associated with diabetic microvascular complications including chronic kidney disease, albuminuria, and retinopathy similarly to HbA_{1c} [30]. The associations of GA and fructosamine with microvascular outcomes were evident in logistic regression models even after adjustment for HbA_{1c}, suggesting that these markers of glycemic control may contribute independent risk information above and beyond the information conveyed by HbA_{1c}. 1,5-Anhydroglucitol was similarly found to be associated with albuminuria and retinopathy in the same study. An animal study involving *db/db* mice reported that normalizing GA significantly lowered collagen IV and albumin excretion and ameliorated the fall in creatinine clearance and the rise in serum creatinine despite persistent hyperglycemia, suggesting that GA may have an important nephropathogenic role that might be therapeutically addressed independently of glycemic status [31]. In a nested case-control study involving women older than 65 years, elevated fructosamine levels of more than 285 μg/L were associated with cardiovascular disease mortality in women without diabetes [32]. Recent studies reported GA as an index that predicted the development of coronary artery disease as well as its severity [33,34]. Another study reported that GA accurately predicted the risk of death and hospitalizations in patients with diabetes mellitus and end-stage renal failure, above that of HbA_{1c} and random serum glucose [35], further illustrating the predictive role of these non-HbA_{1c} biomarkers in cardiovascular outcome and mortality.

With an increasing number of studies demonstrating the unique utility of each of these non-HbA_{1c} biomarkers, it is timely that comparative evaluation of each of these markers be carried out to assess how they can complement each other in the management of diabetes. A recent comparative study indicated that all 4 biomarkers, GA, fructosamine, 1,5-AG, and HbA_{1c}, have a similar degree of correlation with continuous glucose monitor-measured mean glucose (absolute r value = 0.50-0.56) and with hyperglycemic area under the curve at 10 mmol/L [36]. Further larger and prospective studies are imperative to explore the association of these non-HbA_{1c} markers with diabetic complications; morbidity, especially cardiovascular; as well as mortality outcomes. Even more importantly, the question that remains to be answered is whether treatment algorithms using and subsequently targeting these non-HbA_{1c} biomarkers will be associated with a better outcome in diabetic complication rates, morbidity, and mortality in comparison with HbA_{1c}.

One could argue that no marker of glycemia is by itself perfect and that the specific utility of a marker would depend on the specifics of the population under study, the period examined, and the clinical outcome of interest. A perfect biomarker would not only be precise, accurate, and consistent across all different population subgroups, but ideally should also be able to fulfill both a significant and accurate diagnostic and predictive role. The ability to function as a reliable predictor of both micro- and macrovascular outcomes of diabetes will be an invaluable hallmark of the ideal marker of glycemia. Furthermore, changes in glycemic control and risk for diabetic complications in response to available treatment approaches should be reflected in changes of levels of an ideal biomarker; and its utilization should be associated with unequivocal outcome benefits. We can only hope that future scientific endeavors, by providing more data on the factors that could influence the levels of specific biomarkers and by comparatively evaluating the accuracy of available markers in assessing glycemia as well as the clinical utility of available

markers, will bring us a step closer to using as cost effectively as possible a marker of glycemic control or possibly a set of markers, each one possibly having a more appropriate utility in different patient groups at different time points, in the years to come.

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