Editorial

Diagnosis of Gestational Diabetes Mellitus Will Be Flawed until We Can Measure Glucose

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Gestational diabetes mellitus (GDM) is defined by glucose intolerance that is first documented during pregnancy (1). Maternal hyperglycemia that is less severe than that in diabetes mellitus is strongly associated with increased birth weight, risk of Caesarean delivery, and other adverse outcomes (2). Diagnosis of GDM is important because the risk of adverse events is reduced by treatment (3, 4).

Diagnosis of GDM depends exclusively on measurements of plasma glucose. Glucose is typically measured before and at fixed time points after an oral glucose load (5). However, a diagnosis of GDM often is made or excluded based on a single measured glucose concentration above or below a defined threshold (6). By contrast, outside of pregnancy, diagnosis of diabetes requires, in the absence of typical symptoms, documentation of hyperglycemia (by either glucose or hemoglobin A1c) on more than one occasion (7). The requirement for accurate glucose measurements for diagnosis of GDM is heightened by the fact that hemoglobin A1c is not an alternative to glucose measurements for diagnosis of GDM.

A major source of preanalytical error in measuring glucose is loss of glucose from blood specimens through glycolysis occurring primarily in red and white blood cells (8). Glucose is lost from whole blood samples at a rate of 5%–7% per hour at room temperature (8). Thus, loss of glucose at 1 h exceeds the desirable limit of total analytical error for glucose based on biological variation. This preanalytical loss of glucose poses a threat to the diagnostic sensitivity of testing for GDM. Moreover, variation in time to centrifugation of blood samples introduces variability in glucose results with resultant variability in likelihood of diagnosis of GDM.

Sodium fluoride (NaF) is widely used to inhibit glycolysis, but it is inadequate. NaF does not stop glycolysis for the first 2 h or more after sample collection, and during the first 60–90 min the loss of glucose proceeds at the same rate with or without NaF (8, 9). Glucose tolerance testing presents a special problem when the fasting and other samples are held at the point of care (POC) for ≥2 h until completion of the procedure (10).

The AACC and American Diabetes Association (ADA) guideline on laboratory testing in diabetes (8) addresses the handling of samples collected for measurement of glucose in the diagnosis of diabetes. The guideline recommends that samples be immediately immersed in an ice slurry and analyzed within 30 min of collection (8). This is difficult to achieve in routine patient care, and evidence suggests that this recommendation is not always followed in testing for GDM (10, 11).

A study in this issue of *Clinical Chemistry* addresses the problem that glycolysis presents in the diagnosis of GDM (12). In a previous study (10), some of the same authors compared results of GDM testing in which samples were handled either with their institution's usual procedures, including use of NaF-containing blood tubes, or according to the ADA/AACC-recommended procedures (8). The recommended sample-handling approach produced a 2.7-fold increase in the rate of diagnosis of GDM (10). The increase was entirely attributable to control of glycolysis.

Now these authors have evaluated the potential of glucose meters, used at the point of care, to improve the diagnostic accuracy of testing for GDM by minimizing the loss of glucose before analysis (12). Thus, they measured glucose in skin-puncture blood at the POC, with samples collected from patients in the fasting state and at 1 and 2 h after a 75-gram oral glucose load. Venous blood samples were collected at the same times, and handled strictly according the ADA/AACC guidelines; glucose in the venous plasma was measured in a laboratory nationally accredited according to ISO 15189. Linear regression analysis of the POC glucose results vs. the laboratory plasma glucose results was conducted using a randomly selected derivation cohort (n = 102). The resulting equations were used to calculate the predicted laboratory plasma glucose result corresponding to each POC capillary result in the remainder of the cohort (n = 100). For estimation of diagnostic accuracy of the glucose meters in the latter cohort, the predicted venous values were used rather than the actual meter results. For the reference standard for diagnosis of

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GDM, the authors used the glucose concentrations in the venous plasma samples. For both types of samples the same diagnostic criteria (6) were used.

The diagnostic accuracy with the predicted venous plasma results was not as good as might be hoped: The diagnostic sensitivity was 80.4% (95% CI 67.6–89.8) and the diagnostic specificity was 86.4% (72.7–94.8). The positive and negative likelihood ratios for predicting a diagnosis of GDM (made by the reference standard) can be calculated to be 5.9 and 0.23, respectively, corresponding to moderate changes in post-test probability of GDM.

The authors concluded that the study does not support the use of meters "in high-resource settings where measures to inhibit glycolysis are implemented." They suggest that meters may be useful in settings where "measures to inhibit glycolysis are not achievable" (12).

We agree that the results provide limited support for the use of glucose meters for diagnosis of GDM. As the authors acknowledge, the observed diagnostic accuracy cannot be expected in a different setting. The data represent a best-case estimate of diagnostic accuracy: All samples were analyzed by a single meter-operator who used a single device, which was enrolled in an external quality-assurance scheme with professional supervision. Importantly, the equation used to predict venous plasma glucose concentrations from glucose-meter results used data internal to the study. Better results might have been obtained with a different meter, but that points to another danger in concluding that glucose meters can be used for diagnosis of GDM: Meters vary in analytical performance (13).

An alternative to meters was suggested in an earlier study by some of the same authors. That earlier study (11) examined use of blood tubes containing citrate as well as fluoride and EDTA (CFE tubes), which inhibit glycolysis completely or nearly so (14). Compared with usual preanalytical procedures with NaF tubes, use of CFE tubes (without need for an ice-slurry) doubled the diagnostic sensitivity of the oral glucose tolerance test from 42% to 86% ($P < 0.0001$) for diagnosis of GDM, and it produced 100% diagnostic specificity. The likelihood ratio of a negative result with CFE tubes can be calculated to be 0.14. Thus, a negative result implies a >30% decrease in probability of GDM. Using a specificity of 95% (rather than 100%), the positive likelihood ratio is >17. Thus, a positive result connotes a large, >45%, increase in post-test probability of GDM. Based on their findings, the authors recommended that CFE tubes replace traditional NaF-containing tubes in the diagnosis of GDM (11).

Use of CFE tubes might be feasible in both high-resource and some resource-limited settings. The cost of the citrate used in CFE tubes surely is low. Compared with using glucose meters, use of CFE tubes avoids the costs of purchasing glucose meters and reagents, and the expenses associated with training operators and initiating a quality assurance and monitoring program.

Standardized use of CFE tubes for glucose tolerance testing for GDM would minimize or eliminate variability in diagnostic sensitivity of testing for GDM attributable to variable delays in sample processing (9). Standardized use of CFE tubes would also facilitate studies of the epidemiology of GDM, which are at risk of bias and virtually impossible to compare when the variable effects of glycolysis can produce a 2-fold change in the diagnostic accuracy of testing for GDM. Beyond GDM, others [e.g., (14, 15)] have recommended that CFE tubes replace tubes that rely on NaF alone to inhibit glycolysis whenever plasma glucose is measured. An impediment to world-wide use of CFE tubes is the continuing lack of commercial availability of the tubes for clinical use, as is the case in the U.S.

An additional reason to use CFE tubes arises from consideration of the design of the Hyperglycemia and Adverse Pregnancy Outcomes study (HAPO) (2). This multinational undertaking determined the graded relationships between glucose intolerance and clinical outcomes in >23,000 pregnancies. Diagnostic criteria for GDM (6) based on glucose results in the study have been endorsed by the ADA and adopted in several countries (5). In the HAPO study (2), samples for glucose were NaF plasma with blood specimens immediately placed in an ice slurry and held on ice until cells and plasma were separated in a refrigerated centrifuge. CFE tubes provide a convenient means to achieve results that are nearly identical to results on samples that are kept on ice (9, 11). It is unfortunate that CFE tubes are not universally commercially available for clinical use, while tubes with only NaF to inhibit glycolysis persist despite calls over the past decade to replace them with CFE tubes (8, 9, 11, 14).

We congratulate O'Malley and colleagues on their multiple, well-performed studies to define the best practices for diagnosis of GDM. They have highlighted the critical need to address preanalytical factors in measurements of glucose and provided valuable insights into ways to improve outcomes for women and newborns.

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