COMMENT ON LEWIS ET AL.

Management of Hemoglobin Variants Detected Incidentally in HbA_{1c} Testing: A Common Problem Currently Lacking a Standard Approach. Diabetes Care 2017;40:e8–e9

Randie R. Little and Curt L. Rohlfing

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We read with concern the article by Lewis et al. (1) regarding HbA_{1c} analysis in the Vitamin D and Type 2 Diabetes (D2d) Study. Although the article focused on reporting the presence of hemoglobin (Hb) variants after incidental detection of variants by HbA_{1c} high-performance liquid chromatography (HPLC) methods, there was also clear mention of a lack of interference from HbS and HbC with the HbA_{1c} assay used for the study—the Tosoh G8 HPLC. Unfortunately, although the authors claim that there is no interference of these common Hb variants with the Tosoh G8 method, there is clear evidence to the contrary. Our study (2) clearly showed a statistically and clinically significant bias in results from this method with all four common Hb variants (HbAS, AC, AD, AE). This variant interference study was published in early 2016, but the actual sample analysis occurred in early 2015 and the interference may have begun as early as 2014. The NGSP website (3) indicates that this bias is "clinically significant" (defined as $>\pm 7\%$ at 6% and/or 9% HbA_{1c}). The Tosoh G8 showed an actual bias of almost 0.8% HbA_{1c} at the 9% HbA_{1c} level. The recommendation by the NGSP and others is to use a method that does not show interference in order to report an accurate result. This is important in routine clinical care, but it is also essential for important clinical studies such as D2d where 2.2% of participants had an Hb variant.

For some methods, variant interference is an issue that is consistent with the method and does not change over time unless major changes are made to the method. However, with ion exchange HPLC methods, subtle changes in software or reagents can cause changes in the way each variant is eluted off the column and can thus affect the degree of variant interference. Even small statistically significant interferences that are not clinically significant can impact research studies and clinical trials. For example, a recent article (4) concluded that individuals with sickle cell trait (HbAS) had significantly lower HbA_{1c} for any given fasting or postprandial glucose concentration. This conclusion is likely in error given that the HbA_{1c} methods used for the study, although not showing clinically significant interference during the time period in which the samples were analyzed, did show statistically significant biases comparable to the differences reported by the authors. Therefore, the conclusion that HbA_{1c} results in individuals with sickle cell trait are lower than those in subjects without sickle cell trait may be incorrect.

HbA_{1c} measurement has improved tremendously over the years. With improvement in method precision, we are now able to detect small interferences due to Hb variants. These interferences might not always be significant in clinical settings but could still affect clinical studies. More importantly, as in the case of the Tosoh G8, interferences for some methods can change over time and these changes can result in clinically significant interference. Even though Tosoh has been able to alleviate these interferences by changing their software, this new software version is still not available in the U.S. Laboratories and physicians need to be aware of HbA_{1c} method interferences so that accurate results can be reported for patient care and clinical studies.

Duality of Interest. No conflicts of interest relevant to this article were reported.

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University of Missouri School of Medicine, Columbia, MO

Corresponding author: Randie R. Little, littler@health.missouri.edu.

RESPONSE TO COMMENT ON LEWIS ET AL.

Management of Hemoglobin Variants Detected Incidentally in HbA_{1c} Testing: A Common Problem Currently Lacking a Standard Approach. Diabetes Care 2017;40:e8–e9

Michael R. Lewis,[1] Patricia R. Sheehan,[2] Myrlene A. Staten,[3] Lawrence S. Phillips,[4] and Anastassios G. Pittas[2]

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We thank Drs. Little and Rohlfing for their comments (1) regarding our article on the management of incidental detection of hemoglobin (Hb) variants in HbA_{1c} testing (2). We agree that awareness of the potential for interference to affect HbA_{1c} results is essential in research and clinical care. Multiple studies (including that of Lin et al. [3]) prior to the 2016 article by Rohlfing et al. (4) had yielded no evidence of clinically significant bias resulting from HbAS or HbAC variants in Tosoh G8 results. In the Vitamin D and Type 2 Diabetes (D2d) Study, we excluded potential participants with HbAE and HbAD because clinically significant bias had been demonstrated.

In a recent article by Rohlfing et al. (4), HbA_{1c} testing using a Tosoh G8 with software version 5.20 was found to show clinically significant interference (5) in the presence of HbAS and HbAC at the 9% HbA_{1c} level (mean difference with comparative method, 0.76 percentage points for HbAC, 0.79 percentage points for HbAS). Interference at the 6% HbA_{1c} level, which is relevant to D2d given the study's focus on prediabetes, was not found to be clinically significant (0.33 percentage points for HbAC, 0.18 percentage points for HbAS). We acknowledge that "not clinically significant" is not equivalent to "no interference." Nonetheless, any potential interference should not have a meaningful effect on the conduct of the D2d Study because only 37 out of 2,423 participants have HbAS ($n = 27$) or HbAC ($n = 10$) variants, and HbA_{1c} is only one of three criteria used to define prediabetes or diabetes. Moreover, owing to the randomized design, the internal validity of D2d should not be affected.

Changes in assay properties are to be expected over the course of a long-term clinical trial such as D2d, and there have so far been two software updates to the Tosoh G8 used in the D2d central laboratory. The software version (5.20) noted in the article by Rohlfing et al. (4) was in use from June 2014 through December 2015, during which time only eight participants with HbAS or HbAC were randomized. Prior to that period, version 5.10 was used; version 5.23 has been in use since December 2015 and remains in place as we follow our study population. Review of older articles cited on the NGSP interferences web page (6) does not reveal which software versions may have been in use in those studies, and we are unaware of data on possible interferences rising to a level of clinical significance for versions 5.10 and 5.23. To our knowledge, version 5.24 is not yet available in the U.S.

When selecting an HbA_{1c} assay for research, avoiding interference due to rare Hb traits is important and needs to be balanced with other factors. The low coefficient of variation of the ion exchange high-performance liquid chromatography method we chose is desirable for a study such as D2d because it optimizes internal validity.

We agree with Little and Rohlfing (1) that the HbA_{1c} assay is not as straightforward as is sometimes thought and that more work is needed both to understand operational characteristics (especially in individuals with Hb variants) and to define its role as a diagnostic criterion for diabetes. D2d is the first large trial to use HbA_{1c} as one of the inclusion criteria for prediabetes and as a diagnostic criterion for diabetes; therefore, it represents an ideal setting in which to gain insight into these complex issues.

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[1]*Department of Pathology and Laboratory Medicine, University of Vermont, Burlington, VT*
[2]*Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Tufts Medical Center, Boston, MA*
[3]*Kelly Government Solutions for National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD*
[4]*Atlanta VA Medical Center, Decatur, GA, and Division of Endocrinology, Metabolism and Lipids, Department of Medicine, Emory University School of Medicine, Atlanta, GA*

Corresponding author: Michael R. Lewis, michael.lewis@uvmhealth.org.

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