Lower HbA1c seen with sickle trait, but questions remain

Anne Paxton

March 2017—Perhaps unusually for news about clinical diagnostics research, an article in the Feb. 7 issue of *JAMA* created a mild stir with findings that HbA_{1c} results in patients with sickle cell trait, the most common hemoglobin variant in the U.S., may systematically underestimate past glycemia (Lacy ME, et al. 317[5]:507–515).

The article, titled "Association of sickle cell trait with hemoglobin A1c in African Americans," reports on what is likely the largest study to date of the association between sickle cell trait (SCT) and HbA_{1c} for given levels of fasting or two-hour glucose levels among African Americans. Pooling data from two long-term studies—the Coronary Artery Risk Development in Young Adults (CARDIA) study and the Jackson Heart Study (JHS)—the *JAMA* authors looked retrospectively at more than 4,500 participants to conclude that HbA_{1c} was lower in people with sickle cell trait than those without, and that the difference was greater at high glucose concentrations.

National Public Radio segments, stories in the mass media, and Twitter posts surfaced with the news that "Sickle cell trait skews common diabetes test." Because of the scale of the study and its publication in a high-profile journal, "there's been a huge amount of interest," says study coauthor David B. Sacks, MB ChB, chief of clinical chemistry, Department of Laboratory Medicine, National Institutes of Health Clinical Center, and a member of the CAP Chemistry Resource Committee.

The researchers collected data on visits of participants in the CARDIA and JHS studies, scheduled at about five-year intervals, and used generalized estimating equations (GEE) to examine the association of SCT with HbA_{1c} levels, controlling for fasting or two-hour glucose measurements. The study excluded participants without SCT data, without any concurrent HbA_{1c} and glucose measurements, or with hemoglobin variants HbSS, HbCC, or HbAC.

The SCT status of participants—with sickle cell trait defined as the presence of one abnormal allele for HbS—was determined in CARDIA with available DNA samples using single-gene, single-nucleotide polymorphism genotyping and in JHS through whole exome sequencing using data from baseline. Two assays certified in the NGSP (formerly the National Glycohemoglobin Standardization Program), a Tosoh 2.2 and Tosoh G7 (variant mode), were used to measure HbA_{1c} using high-performance liquid chromatography.

Based on this analytic sample—9,062 concurrent measures of fasting glucose and HbA_{1c} levels—the researchers found that in unadjusted GEE analyses, for a given fasting glucose, HbA_{1c} values were statistically significantly lower in those with (5.72 percent) versus those without (6.01 percent) SCT (mean HbA_{1c} difference, –0.29 percent; 95 percent CI, –0.35 percent to –0.23 percent). Adjusting for key risk factors and analyzing 2,001 concurrent measures of two-hour glucose and HbA_{1c} concentration for those with SCT versus those without produced similar findings.

The study authors hypothesize that, although data are limited, the presence of HbS results in a shorter lifespan for red blood cells, leading to less available time for hemoglobin glycation, which in turn may influence the interpretation of HbA_{1c} in relationship to the glucose values they purport to represent. Another possible explanation is that the presence of HbS can result in assay interference with HbA_{1c} measurement techniques.

Interestingly, the findings of the *JAMA* study stand in contrast with those of two previous studies with smaller sample sizes. In one, Bleyer and colleagues looked at 385 African American inpatients, most with diabetes, and, despite higher baseline HbA_{1c} values, did not find that SCT significantly altered the relationship between HbA_{1c} and serum glucose (Bleyer AJ, et al. *Diabet Med.* 2010;27[9]:1012–1016). A second study examined a cohort of 216

African immigrants without diabetes and found no significant difference in the sensitivity of HbA_{1c} by variant hemoglobin status in the detection of prediabetes (Sumner AE, et al. *Diabetes Care.* 2015;38[2]:213–219).

Important factors of the *JAMA* study should not escape attention, Dr. Sacks cautions. "One of the very important ones for clinical laboratories is the test method used to measure HbA_{1c}." In the *JAMA* study, all the assays were done using a single high-performance liquid chromatography method, which is less commonly employed in the U.S. than immunoassay. "So other methods need to be studied to understand the findings. One cannot reach the conclusion that this is true for all HbA_{1c} measurements; you may get different results with other assays."

In fact, he was a coauthor of the Sumner study of African immigrants to the U.S. in which that very thing happened. "We used more than one method to measure HbA_{1c} and we found there was no significant difference in sensitivity of HbA_{1c} in people with sickle trait compared to people without it." Immunoassay, Dr. Sacks notes, is unable to detect that a patient has sickle cell trait before analysis, whereas with capillary electrophoresis and HPLC methods, "you can actually look at the tracing and usually see the variant hemoglobin."

Dr. Sacks

To address the single-method weakness of the *JAMA* study, he says, another retrospective study could still suffice, "as long as there is enough properly stored blood that one can actually analyze it by different methods."

The *JAMA* study is likely to stoke discussion about whe-ther the cutoff for HbA_{1c} should be different in different racial groups, Dr. Sacks says. As the study authors write: "These findings raise the possibility of benefit from incorporating information on hemoglobin variants into clinical guidelines for interpreting HbA_{1c} values for screening and diagnosis of prediabetes and diabetes."

"One of the actually intriguing implications of the study is evidence that's well documented that HbA_{1c} in African Americans born in the U.S. is higher than that of whites. And this has been shown in several studies," Dr. Sacks says.

"What is hotly debated and contested and is unresolved is whether this is clinically meaningful—whether this difference is related to glycemia. Is the higher HbA_{1c} in blacks a reflection of higher glucose or is it independent of glucose? I think that's the critical question and that's not known." The *JAMA* study further complicates the debate by showing that people with sickle cell trait have lower HbA_{1c}. "That makes the clinical findings very difficult—the whole nuance of this is that the implications are potentially large."

Studies in other countries have shown conflicting results. "One study in Asian Indians suggested they may have higher HbA_{1c} than whites, but a study in Japan of about 20,000 people found that whites and Japanese had the same HbA_{1c}." A meta-analysis that combined data from 300,000 people on five continents compared HbA_{1c} to fasting glucose and glucose tolerance tests, he adds, but there wasn't enough information regarding the ethnic composition of participants to reach a conclusion.

The number of people potentially affected by a racial or ethnic component to HbA_{1c} has made the research questions more urgent. "This whole issue really only surfaced when HbA_{1c} became recommended for diagnosis in 2010. People had been using it for monitoring people with diabetes for years and years and nobody raised this

concern," Dr. Sacks points out. He hopes that ongoing studies addressing racial differences might resolve some of the issues. The study authors suggest that future studies that include biracial populations further investigate HbA_{1c} differences by race in relation to hemoglobinopathies. Future studies should also investigate, they say, whether a possible delay in the diagnosis and treatment of prediabetes and diabetes in those with SCT could explain their lower kidney function.

A definitive answer would not be possible without a more precise indication of the average glucose in the blood, obtained by continuous glucose monitoring, for example. "Many of the studies have just looked at one or two fasting glucose values and done a glucose tolerance test, but those only indicate the glucose concentration in the blood at a specific point in time—not 10 minutes before you put the needle in the patient or 20 minutes after you drew the blood—whereas HbA_{1c} reflects a longer period of time, around eight to 12 weeks."

Looking at the broader context, Dr. Sacks says, "For 25 years, HbA_{1c} has been used for monitoring treatment of people with diabetes, and there's very clear evidence that HbA_{1c} correlates very, very well with the risk of microvascular complications, such as blindness and kidney failure, much better than glucose." Tens of millions of patients have benefited from this type of monitoring, he adds.

The available diagnostics for HbA_{1c} are also performing well. "I think the manufacturers have been remarkable in working with the NGSP to improve the assays and reduce the interference from hemoglobin variants." In fact, he says, the recommendation that spurred the 2010 guidelines clearly conveyed that after the many years in which it was advised that HbA_{1c} not be used for diagnosis, it was because the assay had improved that the standard-setting organizations were now recommending its use for diagnosis.

Amid the controversies, the most important thing for laboratorians to keep in mind, Dr. Sacks says, is that "it would be premature to be changing guidelines based on one paper."

"It remains an open question as to whether HbA_{1c} values are significantly different in people with sickle cell trait and whether the cutoffs for diagnosis and perhaps targets for treatment should be changed in these individuals." He hopes that the *JAMA* study will spur further studies that will find the answers that can improve patient care.
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