HbA1c platforms studied for lipemia interference

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February 2019—A forgotten creditor. A poor relation. An envious rival. In the theater, one of these characters often emerges from the woodwork, ready to supply a plot twist just when the protagonist is riding highest. In the health care world, a new study shows, laboratories may be finding that the very popularity of hemoglobin A1c has magnified the underappreciated effect of fats known to interfere with this centrally important diabetes diagnostic. Enter, stage left: Lipemia.

‘Laboratories need to be aware that this interference may occur, especially if they’re using a photometric-based method.’ —Paul Yip, PhD, D(ABCC)

Interferences in general are an interest of Paul M. Yip, PhD, D(ABCC)—in particular interferences that affect front-line chemistry testing. “Many times users are unaware when there may be an interference present that can affect the final result, especially when serum indices are not checked,” says Dr. Yip, division head of biochemistry at Sunnybrook Health Sciences Centre and associate professor in the Department of Laboratory Medicine and Pathobiology, University of Toronto.

Reporting on results of a study he conducted with colleague Michelle L. Parker, PhD, Dr. Yip said in a poster presentation at the AACC annual meeting last July that lipemia interferes differently with HbA1c results depending on the assay method used and the extent of the lipemia (Parker ML, et al. Abstract 288).

To conduct the study, which received funding support from Bio-Rad Laboratories Canada, Drs. Yip and Parker used samples spiked with saline and/or Intralipid to generate triglyceride levels of 0, 5, and 20 g/L to investigate the concentration of Intralipid-sourced triglycerides that may cause significant interference on four commonly used HbA1c analytical methods, and they used clinically lipemic specimens to assess the performance of nine routine HbA1c platforms. “We included essentially all the major manufacturers’ platforms available in North America,” Dr. Yip says.

The platforms studied were: Bio-Rad D-100, Bio-Rad Variant II Turbo 2.0, and Bio-Rad VII HbA2/HbA1c Dual Program; Sebia Capillarys; Beckman Coulter AU; Siemens Dimension Vista; Roche Cobas c501 Tina-quant; Ortho Vitros; and Abbott Architect.

Most immunoassays and the enzymatic method for HbA1c, the study found, are susceptible to negative interference from elevated triglycerides, while chromatographic and electrophoretic methods are resistant. For the

Intralipid-spiked specimens, similar results were obtained; the findings are consistent with an earlier study that also used triglyceride/cholesterol-spiked specimens but assessed fewer assays (Wu X, et al. *Biochem Med [Zagreb]*. 2016;26[3]:353–364).

The assays that involved more of the traditional separation of the specimens were ones that were less affected by lipemia, Dr. Yip says. “Meanwhile, the other methods that used large chemical auto-analyzers that do multiple tests and have non-separation–based assays and any photometric or optical method that uses light” were also thrown off by lipemia. “In the presence of cloudiness or turbidity, the lipids in the sample are obviously going to have an impact on the light,” he says.
To avoid reporting falsely low HbA1c measurements, the study concluded, laboratories should consider evaluating their assay performance for significant interference from clinical lipemia. In addition, the authors said, “Although further investigations are needed, our data suggest that a serum triglyceride threshold of approximately 10 mmol/L may warrant a cautionary note when reporting HbA1c or reflexive testing to a lipemia-resistant platform.”

Hemolysis, hyperbilirubinemia, and lipemia are serum indices, Dr. Yip notes, a general class of interferences that occur in serum or plasma samples and may arise because of the patient’s physiological state or the handling of the samples. While considerable attention has been paid to the ways that hemoglobin variants can interfere with HbA1c, matrix-related interferences like lipemia have been investigated less, he says.

Lipemia’s interference with HbA1c is an under-recognized problem, in his view. “HbA1c is available on many different automated platforms, but not much has been reported in terms of how lipemia can affect results,” Dr. Yip says. “No one has really encountered, at least to my knowledge, a result that was so discrepant that through their troubleshooting and investigation they determined it was due specifically to a lipid interference.”

Lipids in a patient specimen can vary considerably. “They can be produced within the body, they may arise from diet if you have had a fatty meal, and they are heterogeneous in composition, in terms of the size of the lipid particles and the different concentrations of individual lipids. All of those things together can have a very different impact on the analysis.”

When laboratories are reporting on the presence of lipemia, often they are dealing with a serum or a plasma sample, Dr. Yip notes. “Because the plasma and the cells in the blood have been separated, you can see whether or not the sample is clear or if it is cloudy or murky. HbA1c analysis, though, is using a whole blood sample in which everything appears red. It’s difficult if not impossible to tell whether or not lipemia is there.” Adding to that difficulty, “When users are putting a sample into an automated analyzer, they may not even think to look at the specimen. It’s just a matter of loading it on and letting the machine do the testing.”

Major diagnostics manufacturers generally document common interferences well, he says. “Pretty much all of them we found did make a claim in their package inserts as to the kind of interference and whether it was a robust interference.” It’s difficult to replicate lipemia in a manufacturer’s environment, he says. But it can be simulated through Intralipid, a nutritional supplement that is essentially an emulsion of oil and other lipid material. “You can do an artificial setup by adding specified amounts of Intralipid and checking to see if it has any interference on what you are analyzing.” He and Dr. Parker used that technique in their study.

Says Dr. Yip: “HbA1c has taken on such a primary role as a diagnostic test and we’ve been caught up in improving accuracy, reducing imprecision, standardizing the results, and doing a lot of important work to make the assay available and accessible.” Now that those improvements appear to be in place, “we can start setting our sights on some of the less appreciated issues that can have an impact on results.”

In the traditional HbA1c context of diagnosing diabetes, patients would probably see a primary care physician or address the disease on an ambulatory basis, he notes. “Now we see patients going into the ER and ER doctors ordering HbA1c because they want to diagnose diabetes or see what sort of diabetic state the patient is in. In an emergency, you are probably in an acute situation and there are other bodily changes happening at the same time, so you are testing in less than ideal conditions.”

HbA1c is highlighted as the go-to test for diabetes because it doesn't require a fasting specimen. But in these emergency circumstances, a nonfasting specimen is even more likely and the potential for lipemia is going to increase along with that, Dr. Yip says. "For diabetics there is a greater incidence of dyslipidemia as well. If dyslipidemia is present at a sufficiently high level, that can also have an impact on the test result."

Each laboratory needs to determine for itself what its risk of encountering an interference is, he says, and how it is going to mitigate that risk. At University Health Network in Toronto, where Dr. Yip worked previously and where the lipemia study was conducted, he estimated that about 0.2 percent of, or one in 500, samples were significantly lipemic. The method that the UHN laboratory used was not affected by lipemia. "But for any laboratory using a routine auto-analyzer, many if not all of the major chemistry auto-analyzers can do a serum indices check, which could give a crude estimate of how much lipemia might be present."

Dr. Yip sees immediate clinical implications for this study. "That's because we appreciate now that A1c has to perform so tightly, so well, the total allowable error is constantly getting smaller and smaller, and my feeling is that it will continue to tighten. We have various guidelines that specify diagnosis of diabetes at an A1c of 6.5 percent. Now we have to be very sure that that 6.5 percent is as accurate as possible. If you have significant lipemic interference with one of the methods and an error that can falsely lower results, you could be missing that diagnosis of diabetes."

'Laboratories should take note of this and discuss it with their clinicians and determine what the impact is on their patient population.' —James Nichols, PhD, D(ABCC)

It's not surprising that the chemistry analyzers that use a spectrophotometer, except for one of the immunoassays, showed a significant interference by lipemia in samples in the study, says James Nichols, PhD, D(ABCC), medical director of clinical chemistry and point-of-care testing at Vanderbilt University Medical Center and professor of pathology, microbiology, and immunology, Vanderbilt University School of Medicine.

"Any of the spectrophometric methods that we use in chemistry can be interfered with by lipids, by bilirubin, or by hemolysis. The HbA1c analyzed by HPLC or capillary electrophoresis did not show this problem because there you are separating hemoglobin from the constituents of plasma and you separate the different types of hemoglobin on those columns or the capillary. If you have high triglycerides or high lipids, those interferences are going to wash out in the solvent front at the beginning of the run, and then later you'll see the separation of the hemoglobins."

"With the chemistry analyzers, you are mixing the patient's whole blood with the reagent from the manufacturer, and the reaction is occurring in that same cuvette where the detection occurs. Everything is in the same cuvette,

and you're shining light through it, so if you have an interfering substance like lipids, it is going to interfere with that detection if it is the right wavelength." The one method may be using a different wavelength than the other analyzers, he says, and thus there may be less interference for that reason.

Can results from different platforms be comparable then? That is the issue, in Dr. Nichols' view. "If you are seeing a negative effect or impact, as Dr. Yip was showing with the triglycerides, it is significant for diabetics because most diabetics have problems with glucose metabolism. Because they are not metabolizing glucose, they need to metabolize protein and triglycerides or lipids for energy sources."

Many people with diabetes have high cholesterol or high triglycerides. "Because of that, they are going to show, with these particular methods, a decrease in HbA1c. The HbA1c will make them look more compliant or better than they actually are," Dr. Nichols says. "Their estimated average glucose over the past couple of months is going to look a little better than their actual average glucose. And they would be undertreated, in essence."

If a laboratory is monitoring a patient over time with a chemistry platform that is falsely showing a decrease in HbA1c with triglycerides, he says, that is one thing. "But if you are monitoring a patient over time you are probably going to see a trend of increase or decrease overlaid with this interference. If you don't switch methods, you can probably trend a patient over time because their lipids are not going to significantly change, unless there's treatment with lipid-lowering drugs. And we've been managing patients currently pretty well based on these different methods."

A visual inspection for lipemia in the laboratory is common with the automated platforms and chemistry analyzers, Dr. Nichols says. "We used to look at every sample visually and give an assessment of lipemia. Now, a visual inspection is either automated or it is ordered as a part of the chemistry panels. And we append comments on interferences at different levels."
He suspects laboratories that are using the chemistry platforms studied by Drs. Yip and Parker can do a similar type of commenting. "If it was above a particular level that was significant, they would run a lipemia index and append a comment or note that the result may be falsely decreased because of the increase in lipids."

Whether clinicians will notice is a different matter. "It depends on the electronic medical record system. A lot of times we append comments. Up front on the first EMR screen, you'll see a number with an asterisk or a little arrow, but unless the clinicians click on the results on this EMR screen, they don't see the comment. So, there can be issues with missing an important interference comment—and with a magnitude that is clinically significant."

In addition, since the benefit of HbA1c is that the patient does not have to fast before the test, patients may have eaten just before the test and may have higher triglycerides, creating an even larger decrease in their HbA1c results.

High patient triglycerides are common enough to be a concern, Dr. Nichols says. "The study showed a pretty significant difference. The results were negatively biased by 10 percent and 25 percent at 5 g/L and 20 g/L of triglycerides.

"If you are looking at a 10 percent to 25 percent decrease in a result and you are sitting just above abnormal at an HbA1c of seven or eight, a 25 percent decrease will take you down to six, which would be considered intermediary, or even lower into the normal range. That could lead to incorrect treatment or a missed diagnosis." This is more important for less serious cases of diabetes and maybe type 2 diabetes, for which clinicians screen patients in their physical exams, he adds, because if a person is widely out of range with their glucose, they will have HbA1cs of 10, 11, or in the teens. "A 10 percent decrease there is not going to be missed."

Separately, there is already concern about using the chemistry methods for HbA1c because of the risk of missing hemoglobinopathies. "In other words, if you have a patient with sickle cells or high prevalence of hemoglobin variants in your patient population, you should be cautiously using a chemistry analyzer in the first place because the number you get won't tell you that you have a hemoglobin variant, unless the patient knows they have a variant. So that chemistry analyzer number is going to be misleading for HbA1c to start with, depending on the

type of hemoglobin variant and the specific method." Independent of a direct interference, the presence of some hemoglobin variants can increase red cell turnover, affecting HbA1c levels.

Most of the chemistry methods' package inserts list the common interferences, including hemolysis and triglycerides. "That factors in when they are developing the reagents and validation studies as part of the FDA submission data," Dr. Nichols says, although, he adds, apparently the study showed that some of the manufacturers don't have this data in their inserts.

The study results do not mean that laboratories should think right away about switching methods if they are showing a bias, Dr. Nichols says. Instead, "Laboratories should take note of this and discuss it with their clinicians and determine what the impact is on their patient population."

In response to the study, Jeannine Holden, MD, Beckman Coulter's chief medical officer, says lipemia is potentially an issue for any assay with a turbidimetric readout, not just HbA1c, and the study results Dr. Yip and colleague obtained are consistent with Beckman Coulter's instructions for use for its HbA1c assay.

HbA1c assays are challenging in many respects, she says, and each of the common assay types has its benefits and drawbacks. "Immunoassays are generally more resistant to variant hemoglobins, so they may be preferable in areas with higher prevalence, and they don't require a separate, dedicated instrument."

Lipemia is underappreciated as a source of interference, Dr. Holden says. "In general, clinician awareness of lipemia as a potential interfering substance for assays is low, so laboratorians need to consider it when reporting any turbidimetric assay, not just HbA1c. Clinicians may also be unaware of the impact of red cell turnover on HbA1c results regardless of the assay type," she adds, "and unlike lipemia, accelerated red blood cell turnover may be difficult to detect."

Does Roche recommend the cautionary note suggested in the study? Günter Trefz, PhD, head of homogeneous immunoassay development, R&D Germany, Roche Diagnostics, points to the cautionary note on the Cobas c501Tina-quant package insert and notes that the limitations section clearly states: "Lipemia (Intralipid): No significant interference up to an Intralipid concentration of 600 mg/dL. There is poor correlation between triglycerides concentration and turbidity."

"According to internal statistical data, the number of samples with an L-index greater than 200 mg/dL is less than 0.1 percent," Dr. Trefz tells CAP TODAY. The number of samples with L-index greater than 500 mg/dL has been shown to be less than 0.02 percent in another study (Mainali S, et al. *Pract Lab Med*. 2017;8:1–9). "Therefore, it is rare to have samples with an L-index at or above 600 mg/dL."

The company refers users to the NGSP website (ngsp.org/factors.asp) for full details, but says, "High-performance liquid chromatography methods for determination of HbA1c may be resistant to lipemia, but HPLC users must be aware of potential hemoglobin variant interference which exists at a higher prevalence and leads to inaccurate % HbA1c values."

Roche also raises a question about the difference between the study's methodology and CAP Surveys. "In this study, there appears to be a problem with how zero lipemia samples were measured. The baseline for zero lipemia samples appears to have been measured with an HPLC or CE method, resulting in bias with the Roche Tina-quant method. This finding is not seen in CAP Surveys, where the Roche Tina-quant method shows no significant bias. The correct procedure to detect lipemia interference would be to baseline each assay individually with the zero lipemia sample."

Dr. Yip is continuing his research on lipemia interference by studying larger numbers of HbA1c samples on the various platforms. In the meantime, he says, "Laboratories need to be aware that this interference may occur, especially if they're using a photometric-based method. And they should talk to the manufacturers about how they can approach the potential interference."

Anne Paxton is a writer and attorney in Seattle.