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Q&A

Perspectives on the Changing Landscape of Measuring Cardiovascular Risk Related to LDL

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The clinical laboratory plays a critical role in the assessment of atherosclerotic cardiovascular disease (ASCVD)[9] risk. Since the 1970s, this laboratory assessment has been primarily through tests in the lipid panel, which included cholesterol, triglycerides (TG), and HDL cholesterol (HDL-C) based on fasting serum or plasma specimens. Until the advent of direct LDL cholesterol (LDL-C) tests approximately 20 years ago, LDL-C was almost exclusively estimated by the Friedewald equation (valid when TG <400 mg/dL or <4.5 mmol/L), and to date the vast majority of LDL-C is still calculated. This testing paradigm has undergone substantial changes over the past decade, with the emergence of new research and guidelines recommending alternate approaches for the laboratory assessment of ASCVD risk.

As early as 2009, various clinical laboratory associations started recommending the use of a nonfasting specimen for routine lipid panels as a result of several studies supporting its use in cardiovascular risk assessment. In 2013, Martin and coworkers reported a new equation for estimating LDL-C that was more accurate than the Friedewald equation when compared to ultracentrifugation method (also known as β quantification). This new equation was reported to overcome the poor performance of the Friedewald equation at TG >150 mg/dL (>1.7 mmol/L) and at low concentrations of LDL-C (<70 mg/dL or <1.8 mmol/L), which may now be more common and clinically relevant owing to the use of more effective lipid-lowering therapy. Not all studies, however, have shown a significant difference between the old and new LDL-C equations when compared to preparative ultracentrifugation, the gold standard method for measuring LDL-C. Nevertheless, many clinical laboratories and at least one major provider of diagnostic testing services in the US switched to the new equation in 2017.

In 2018, the American College of Cardiology and the American Heart Association (ACC/AHA) published new guidelines on the management of blood cholesterol. Notably, these guidelines endorsed the use of nonfasting specimens; classified increased concentrations of lipoprotein(a), high-sensitivity C-reactive protein, TG, and apolipoprotein B (apoB) as risk-enhancing factors; and recommended their consideration in intermediate ASCVD risk patients. They also recommended the use of the Martin equation (also known as the Martin–Hopkins equation) for specimens with low LDL-C. Additionally, in 2017 the US Food and Drug Administration cleared a new direct test for small dense LDL-C (sdLDL-C), which in 3 large clinical studies (Framingham Offspring Study, Atherosclerosis Risk in Communities Study, and Multi-Ethnic Study of Atherosclerosis) was found to be potentially useful for ASCVD risk prediction. To address all these new developments, we invited a group of experts consisting of cardiologists, epidemiologists, clinical researchers, and clinical chemists to share their views on these topics.

Which equation are you currently using to estimate LDL-C and under what circumstances? When do you recommend an alternative test like a direct LDL-C?

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[9] Nonstandard abbreviations: ASCVD, atherosclerotic cardiovascular disease; TG, triglycerides; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; ACC/AHA, American College of Cardiology/American Heart Association; apoB, apolipoprotein B; sdLDL-C, small dense LDL-C; VLDL, VLDL cholesterol; CVD, cardiovascular disease.

Børge G. Nordestgaard: With nonfasting plasma samples, we use the Friedewald equation to estimate LDL-C if plasma TG are <352 mg/dL (4 mmol/L). If plasma TG are higher, we measure LDL-C with a direct assay.

Harvey W. Kaufman: LDL-C is part of the standard lipid panel, which includes direct measurement of total cholesterol, HDL-C, and TG. Based on the Friedewald equation, the TG (in mg/dL) divided by 5 is used to estimate the VLDL cholesterol (VLDL-C, mg/dL). This VLDL-C estimate along with direct HDL-C is subtracted from the total cholesterol to yield the calculated LDL-C.

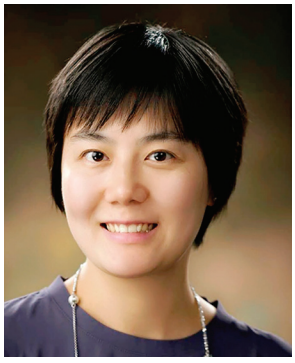
We recognized that the Friedewald equation that had served us well for 45 years often underestimated LDL-C. Further, the Friedewald equation was not intended to calculate LDL-C for the increasingly observed patients with extremely low LDL-C concentrations. Quest Diagnostics was among the first laboratories to adopt the Martin equation in September 2017. The Martin equation is valid across a broad range of LDL-C and TG concentrations. Both fasting and nonfasting specimens are acceptable, thus making specimen collection easier for patients and medical practices.

The direct measure of LDL-C may seem like the best approach. However, it comes with a cost, and all the direct methods included in a study failed to meet the National Cholesterol Education Program total error goals for nondiseased individuals due to lack of specificity toward abnormal lipoproteins.

Samia Mora: When I am evaluating patients clinically, I use the LDL-C result that is provided by the clinical laboratory. Clinicians do not have the time to calculate LDL-C during their time-pressured clinical care, hence the important role that the clinical laboratory can play in providing optimal patient care. However, I do calculate non-HDL-C, as it is often still not provided by many clinical laboratories, despite multiple guideline recommendations that laboratories should also provide non-HDL-C results. Currently, most clinical laboratories provide the Friedewald equation for LDL-C when TG

are <400 mg/dL (4.5 mmol/L) and either provide a direct LDL-C or provide a missing value for the LDL-C when TG are equal or >400 mg/dL (4.5 mmol/L).

I do not routinely recommend direct LDL-C measurement. We conducted a large prospective study among 27 000 participants in the Women's Health Study that found similar results for predicting cardiovascular risk whether LDL-C was measured directly (with a homogeneous assay) or calculated by the Friedewald equation, although the results of the direct LDL-C were lower than the calculated Friedewald LDL-C by about 5–10 mg/dL (0.13–0.26 mmol/L). Most epidemiological studies have used the Friedewald equation for LDL-C. It used to be thought that direct LDL-C is better for nonfasting lipid tests, but we now know from many studies that nonfasting and fasting LDL-C results (by Friedewald or other methods) are very similar. Also, there are many direct LDL-C assays, and some of these direct assays provide discordant or even erroneous results. It would be helpful if the laboratory reporting a direct LDL-C result also includes the assay method.



Jing Cao: Currently we use the Friedewald equation to estimate LDL-C, and we have built the table from Martin et al. in our chemistry analyzer middleware. In patients with LDL-C below 100 mg/dL (calculated from Friedewald) and TG between 150 and 399 mg/dL (1.7 and 4.5 mmol/L), LDL-C from both the Friedewald and Martin equations will be reported. When TG exceeds 400 mg/dL (4.5 mmol/L), no calculation of LDL-C will be provided, and a direct LDL-C immunoassay will be recommended to clinicians.

Jeffrey W. Meeusen: We use the Friedewald equation for both fasting and nonfasting specimens when TG <400 mg/dL (4.5 mmol/L). If nonfasting TG are >200 mg/dL (2.3 mmol/L) for men or >175 mg/dL (2.0 mmol/L) for women, we recommend a fasting specimen. We do not endorse direct LDL-C for CV risk assessment. LDL-C measured by preparative ultracentrifugation in the context of a full lipoprotein profile is recommended for cases of unusual dyslipidemia with clinical sequelae.

Why did you choose to implement (or not) the new equations for calculating LDL-C, such as the Martin equation?

Børge G. Nordestgaard: We have used the Martin equation for research, but not yet for routine LDL-C estimation. We may implement the Martin equation in the near future; however, it is more complicated to use than the simple Friedewald equation. Also, for most clinicians and for the majority of patients, the added value of the more accurate LDL-C estimation by the Martin equation seems minor.

Harvey W. Kaufman: The errors in the Friedewald equation have been described for 3 decades. More recent studies have highlighted the same issue. Thus, the opportunity to implement a better approach was aligned with our vision of empowering better health through insights.

Samia Mora: As a clinician and a cardiologist, I rely on the clinical laboratory to provide me with the LDL-C result. Some laboratories are now using the Martin equation, which provides a more nuanced and generally better LDL-C calculation than the Friedewald LDL-C calculation, with the added advantage that the Martin equation can also be used when TG are ≥400 mg/dL (4.5 mmol/L) and when LDL-C is low. Nonetheless, for most patients, the LDL-C results are similar with the Friedewald or Martin equations, unless the TG are increased or the LDL-C is low, in which case the Martin equation is more accurate than ultracentrifugation. On the other hand, for CV risk assessment, total and HDL-C are used in guideline-recommended risk equations (instead of LDL-C).

Jing Cao: We were always concerned about the inaccuracy of Friedewald equation, particularly in our pediatric populations, in which fasting is not strictly followed or required. We have compared LDL-C calculations to the reference method, β quantification, in our own patient population, and found that the Friedewald equation substantially underestimated LDL-C concentrations below 100 mg/dL (2.6 mmol/L) in the presence of increased TG. We have thus derived our algorithm accordingly reporting LDL-C from 2 equations.

Jeffrey W. Meeusen: The improvement in LDL-C estimation is marginal and not likely to affect patient care decisions. Furthermore, non-HDL-C is readily available, has similar or better clinical performance than LDL-C, and lacks the limitations ascribed to both the Friedewald and Martin equations. Finally, the Martin equation requires a license; if patients are being asked to cover the cost for an LDL-C, then they deserve an actual measurement.

What do you think about the utility of reporting non-HDL-C, particularly in light of the new 2018-AHA/ACC guidelines?

Børge G. Nordestgaard: I favor reporting non-HDL-C and have in fact started a process to implement its reporting together with a standard lipid profile at all Copenhagen laboratories. In my own laboratory at Copenhagen University Hospital we now always report non-HDL cholesterol as part of a lipid profile. That said, non-HDL-C should preferably be reported together with its 3 components of LDL-C, remnant-C (VLDL-C) and lipoprotein(a) [roughly 30% of lipoprotein(a) total mass is due to cholesterol]. This is because these 3 lipoprotein fractions possibly influence risk of cardiovascular disease by somewhat different mechanisms. The genetic risk factor lipoprotein(a) should be measured once in all individuals having lipid profiles done to evaluate cardiovascular risk, as recently recommended by the 2019 European dyslipidemia guidelines.

Harvey W. Kaufman: At the urging of Dr. Nader Rifai, following National Cholesterol Education Program, Adult Treatment Panel 3, Quest Diagnostics included calculation of the non-HDL-C with the standard lipid panel.

The 2018 AHA/ACC Guidelines include as "Risk-Enhancing Factors for Clinician–Patient Risk Discussion," primary hypercholesterolemia as defined as LDL-C of 160–189 mg/dL (4.1–4.8 mmol/L) or non-HDL-C of 190–219 mg/dL (4.9–5.6 mmol/L). Relying on LDL-C alone may be misleading. For example, the growing population of individuals with abdominal obesity, metabolic syndrome, or diabetic lipid disorders often have increased TG, low HDL-C, and nonincreased calculated LDL-C. These patients produce highly atherogenic lipoproteins such as VLDL and intermediate-density lipoprotein as well as sdLDL particles.

For this reason, non-HDL-C is a better marker of cardiovascular disease (heart events and stroke) in both primary and secondary prevention studies.

The 2018 ACA/AHA Guidelines also provide for the categorization of non-HDL-C for children.

Samia Mora: All clinical laboratories should report non-HDL-C. Although it is easy to calculate (total minus HDL-C), most clinicians are just too busy to calculate the non-HDL-C, and this is where the laboratories can play an important role. In most studies, non-HDL-C is a better lipid measure of cardiovascular risk than LDL-C, because it incorporates the cholesterol from other atherogenic lipoproteins not just from LDL. This number is

particularly relevant for patients with cardiometabolic risk factors such as increased TG, diabetes, prediabetes, or the metabolic syndrome.

Jing Cao: Non-HDL-C is included in the lipid panel report at Texas Children's Hospital. It is especially useful in the setting of nonfasting screening and in pediatric populations, as indicated in the 2011 NHLBI Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents.

Jeffrey W. Meeusen: Non-HDL-C has been repeatedly shown to have clinical value equal or superior to LDL-C. The use of non-HDL-C as a biomarker of cardiovascular disease (CVD) risk was a recommendation by the National Cholesterol Education Program Adult Treatment Panel and has been reported by many labs, including my own, for over a decade. The inclusion of non-HDL-C as an alternative risk measurement equal to LDL-C in the 2018 guidelines has been well received by clinicians and laboratorians alike, and in many ways is acknowledging routine practice that was omitted in the 2013 guidelines. It is a simple calculation that our clinicians find useful.

How has the use of apoB test changed in your laboratory since the new 2018 AHA/ACC guidelines have recommended it for consideration in intermediate CVD risk patients? If it hasn't changed, why not?

Børge G. Nordestgaard: Scientifically, I favor testing for apoB. However, we do not offer apoB test for routine use yet as (*a*) it appears to offer only slightly improved risk estimation compared with non-HDL-C, (*b*) it is unlikely to offer improved risk estimation above its 3 components of LDL, remnants, and lipoprotein(a) combined, (*c*) it is a complicated task to educate common doctors and patients about what apoB is and why it should be measured, and (*d*) apoB measurement comes at an extra cost compared with a standard lipid profile including calculated LDL-C, calculated remnant-C, and calculated non-HDL-C. However, as now both the 2018 US cholesterol guidelines and the 2019 European dyslipidemia guidelines recommend its use, we may start offering apoB measurements at Copenhagen University Hospital in the near future for expert doctors. In Denmark, patients and doctors do not pay for the test requested, as these are automatically paid through taxes. It is therefore important to make sure that different tests with similar information are not ordered on all patients.

Samia Mora: It is too early to see a difference. It will probably take several years to train and educate clinicians regarding the clinical utility of apoB. In a large study that we recently conducted, the Women's Health Study, apoB was particularly helpful among patients with multiple cardiometabolic risk factors and was better than the LDL-C or the non-HDL-C. Among these patients with cardiometabolic risk factors, there is often a "discordance" noted between the concentration of apoB (increased) and the concentration of LDL-C or non-HDL-C (often not increased or low). Hence, the apoB concentration can capture risk information that is missed by the LDL-C or even by non-HDL-C.

Here, the laboratories can play an important role if they also provide clinically useful apoB cut points for the clinicians, most healthcare providers are not familiar with clinical cut points for apoB, but they are very familiar now with the LDL-C cut points. ApoB can also be very helpful for clinical diagnosis of dyslipidemias including type III dyslipoproteinemia. The recent 2019 European dyslipidemia guidelines provide clinical cut points for apoB in addition to LDL-C and non-HDL-C.

Jing Cao: The recommendation on apoB in children and in women at gestational stage has not changed markedly since the new guideline. Therefore, we have not observed major changes in the use of apoB. In our institution, apoB is a send-out test, and it continues to be ordered by physicians for patients at intermediate ASCVD risk.

Jeffrey W. Meeusen: ApoB is more standardized and better defined than LDL-C. We have not noticed much change in order volume or provider interest; however, the AHA/ACC endorsement of apoB as a risk indicator is a major step forward in modernizing routine lipid assessment.

Have you noticed any effect of the new position statement by the 2018 AHA/ACC guidelines that nonfasting samples are suitable for the initial lipid screening? In your view, what are the pros and cons of this recommendation?

Børge G. Nordestgaard: In Denmark we introduced nonfasting lipid testing in 2009, and today essentially all laboratories use nonfasting samples. Advantages of nonfasting compared with fasting lipid panels include (*a*) nonfasting better captures the lipid and lipoprotein concentrations present for the majority of a 24-h cycle, (*b*) nonfasting avoids problems with hypoglycemia, particularly in those with diabetes, (*c*) nonfasting makes blood sampling simpler for patients, laboratories, and clinicians alike, (*d*) nonfasting is evidence-based, whereas use of fasting lipid profiles simply represents tradition of "what we now have done for so many years," (*e*) time and cost savings for the patient because the need for a return visit for blood sampling is eliminated, and (*f*) it will be easier for patients to participate in randomized trials of lipid-lowering drugs. I see no disadvantage for using nonfast-

ing lipid panels, as this represent the physiological normal condition for humans.

Harvey W. Kaufman: Quest Diagnostics has supported nonfasting samples as an alternative to fasting for many years. The European medical community has embraced nonfasting patient preparation in part because components of the lipid panel correlate better on fasting patient preparation-collected specimens. The TG concentration is typically approximately 25 mg/dL (0.3 mmol/L) higher with nonfasting than fasting.

Samia Mora: For cardiovascular risk screening and prediction, there is now convincing evidence that a nonfasting lipid panel is at least as good as a fasting lipid panel. We recently published a large prospective study from the Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm trial that found that nonfasting lipids were similar to fasting lipids measured 4 weeks apart on the same participants in predicting incident cardiovascular events. In addition, we found very high concordance (≥95%) of fasting and nonfasting lipids for classifying participants into appropriate cardiovascular risk categories for consideration of statin therapy. Finally, nonfasting and fasting lipids provided similar results even among the population that was treated with statin therapy. Nonfasting lipids can facilitate high-quality clinical care, and patients really prefer it. Not only is it easier and a more accurate reflection of our lipid state (because most of the time we are not fasting), but it is also safer because fasting can result in clinically meaningful hypoglycemic events among elderly patients and those with diabetes.

Jing Cao: It has always been a challenge to enforce the 8-h fasting requirement for lipid screen. Physicians in our preventive cardiology clinic have been following the 2011 NHLBI Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, that is, to screen for dyslipidemia with nonfasting samples, followed by confirmative diagnosis using fasting samples. In the primary care setting, however, <50% of pediatricians are ordering lipid screen tests. The recommendation of nonfasting lipid screen has definitely advanced more widespread adoption of the lipid screen strategy.

Jeffrey W. Meeusen: We implemented nonfasting blood draws following their endorsement in the 2013 AHA/ACC guideline. So far it has been a positive experience for patients and providers. In most cases, TG are <200 mg/dL (2.3 mmol/L) and no additional testing is necessary.

Now that a Food and Drug Administration–cleared high-throughput test compatible with standard clinical chemistry analyzers is available for sdLDL-C, how do you think it will change LDL testing for assessment of CVD risk?

Børge G. Nordestgaard: High concentrations of sdLDL-C likely will supplement the information provided by a standard lipid profile, particularly in overweight and obese individuals and in those with metabolic syndrome and high plasma TG. Use of this new automated test may increase further if and when even more high-quality studies are published that document improved predictive ability of sdLDL-C above conventional LDL-C; however, the extra cost of adding this lipid test to a standard lipid panel needs to be considered.

Harvey W. Kaufman: LDL-C is a good but not ideal marker of cardiovascular disease risk. Many or most people with CVD events may have had "good" lipids based on the standard lipid panel. A single LDL particle is about 220–275 Å in diameter, typically transporting 3000–6000 fat molecules/particle and varying in size according to the number and mixture of fat molecules contained within each particle. Particle number allows identification of those patients who have controlled "good" lipids; however, a high particle number indicates that the lipids are not "good" and CVD risk is greater than the standard lipid panel would suggest. Similar information can be obtained through measuring apoB. Family history, age, smoking history, diabetes, obesity, and other important factors affect cardiovascular disease risk. Many people have one or more risk factors. Among clinical laboratory factors, high-sensitivity C-reactive protein and other inflammatory markers provide insights that lipids do not. LDL and HDL subparticles provide a more comprehensive picture of the CVD risk, and as markers they can be used to evaluate the effect of therapeutic interventions and lifestyle changes.

Samia Mora: I don't think it will change LDL testing. In the Framingham Offspring Study, sdLDL-C concentrations using this assay were not significantly different among CVD cases compared with controls. In the Multi-Ethnic Study of Atherosclerosis study, sdLDL-C was not associated with CVD risk among patients with diabetes or impaired fasting glucose. In the Atherosclerosis Risk in Communities study, sdLDL-C was not significantly associated with risk for incident coronary heart disease after further adjustment for apo B or Friedewald LDL-C. Furthermore, our prior work from the Multi-Ethnic Study of Atherosclerosis study and other studies since then have demonstrated that the relationship between sdLDL and CVD risk is confounded (explained) by the concentration (number) of LDL particles. Namely, that individuals with sdLDL may have increased CVD risk, but that risk is explained by these individuals having a greater number of atherogenic particles (e.g., greater apoB or LDL parti-

cle concentrations). All LDL particles are atherogenic, not just the sdLDL. Indeed, patients with familial hypercholesterolemia often have large cholesterol-rich LDL particles. Hence, we should focus our efforts on reducing all atherogenic particles (i.e., apoB), regardless of the size of the LDL particles.

Jing Cao: There have been multiple lines of evidence supporting the role of sdLDL-C as an independent risk factor of ASCVD. The availability of an automated high-throughput sdLDL-C assay offers clinicians a convenient way to identify individuals who are at high risk. However, there are no current guidelines pointing to its utility in the general population.

Jeffrey W. Meeusen: There is a high correlation between concentrations of sdLDL-C, LDL-C, and apoB. Every study that has adjusted models for LDL-C or apoB has reported that sdLDL-C is no longer significantly associated with risk of CVD. The Atherosclerosis Risk in Communities study explicitly states that sdLDL-C was not significantly associated with risk for incident coronary heart disease after further adjustment for other lipid risk factors, such as LDL-C, apo B, or total cholesterol. The Multi-Ethnic Study of Atherosclerosis study reported sdLDL-C performed similarly in dichotomous LDL-C categories when adjusted for HDL-C and TG but did not report on adjustments for total cholesterol, LDL-C, or apoB as continuous variables. The Framingham Offspring Study did not report any adjustments.

Despite the Food and Drug Administration clearance, there are still no data to support that sdLDL-C provides independent risk stratification value over LDL-C or apoB.

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