Estimating LDL ApoB: Infomania or Clinical Advance?

Epidemiological and clinical studies have consistently demonstrated that increased concentrations of LDL cholesterol in plasma are associated with increased risk of atherosclerotic cardiovascular disease (CVD).1 The reference method for LDL cholesterol is β-quantification, a method developed by the CDC that requires ultracentrifugation and is accordingly labor intensive, time consuming, and expensive. Thus, in routine clinical laboratory practice, LDL cholesterol is estimated by the Friedewald formula (1). This equation requires the measurement of total cholesterol, HDL cholesterol, and triglycerides, together with a calculation factor that estimates the concentration of cholesterol in very-low-density lipoprotein (VLDL). However, this formula is not valid for nonfasting patients, when plasma triglyceride concentrations are ≥4.5 mmol/L (400 mg/dL), in familial dysbetalipoproteinemia, or when there is abnormal VLDL composition. Direct measures of LDL cholesterol are available, but they are not standardized and are expensive.

Apolipoprotein B (apoB), a large amphipathic glycoprotein, plays a central role in human lipoprotein metabolism (2). The APOB gene is located on chromosome 2 and produces, via a unique mRNA editing process, two forms of apoB in circulating lipoproteins, apoB-48 and apoB-100 (3). ApoB-48 is the truncated form of apoB-100 consisting of the N-terminal 48% of full-length apoB-100. ApoB-48 is synthesized in the intestine and is essential for the formation and secretion of chylomicrons. ApoB-100 is synthesized in the liver and is an essential structural and functional component of VLDL and its metabolic products, intermediate-density lipoprotein (IDL) and LDL, being the ligand for the LDL receptor.

Essentially all circulating apoB is associated with lipoproteins, and unlike most other apoproteins, apoB cannot exchange freely among lipoprotein particles (2, 3). VLDL, IDL, LDL, and lipoprotein each contain one molecule of apoB-100. Similarly, chylomicrons and their remnants contain a single molecule of apoB-48. Hence, plasma apoB concentrations reflect the total number of atherogenic particles present in the circulation (4, 5). Moreover, like LDL cholesterol, an increased plasma concentration of apoB has been shown to be key risk factor for the development of atherosclerotic CVD.

In a series of prospective epidemiological studies, plasma apoB has been shown to be superior to LDL cholesterol, and has been the topic of recent reviews (5, 6). The largest prospective study is the Apolipoprotein-related Mortality Risk (AMORIS) study (7), in which 175 553 individuals from Sweden were recruited and followed for an average of 5.5 years. The relationships between fatal myocardial infarction, apoproteins, and other lipid measures were examined in multivariate analyses (after adjusting for age, total cholesterol, and triglycerides), and apoB, apoA-I, and apoB/apoA-I ratio were all significant predictors of myocardial infarction in both sexes. Moreover, apoB was more significant than LDL cholesterol in prediction of myocardial infarction in both men and women. ROC analysis showed that apoB had greater sensitivity and specificity than LDL cholesterol as a predictor variable in both sexes, especially in those with normal/low LDL cholesterol. Importantly, the results from several large statin trials show that change in plasma apoB concentrations in response to treatment may be a better predictor of cardiovascular benefit than change in LDL cholesterol (5, 6). Achieving a target level of apoB may therefore be a more important therapeutic objective than a target level of LDL cholesterol.

In routine clinical laboratory practice, total apoB is measured in plasma, by immunoturbidimetric or immunonephelometric methods, thereby avoiding ultracentrifugation (8, 9). The measurement of apoB has been standardized by the IFCC/WHO, is automated, and can be performed on nonfasting samples (10). Although apoB-48 may be recognized by apoB-100 antibodies owing to cross-reactivity, apoB-100 is by far the predominant apoB molecule (>90%) present in plasma, even in the nonfasting state. However, light-scattering techniques can be unreliable in lipemic samples from hypertriglyceridemic individuals.

Increased concentrations of LDL apoB may identify patients with greater numbers of LDL particles in whom atherosclerotic CVD risk is increased (9). These patients may not necessarily have increased concentrations of LDL cholesterol. Unfortunately, LDL apoB cannot be calculated by a process equivalent to the Friedewald formula. Moreover, direct measurement of LDL apoB requires ultracentrifugation, making it labor intensive, time consuming, and expensive, limiting its

1 Nonstandard abbreviations: CVD, cardiovascular disease; VLDL, very-low-density lipoprotein; apoB, apolipoprotein B; IDL, intermediate-density lipoprotein.
availability to tertiary referral or research laboratories. Because of this, equations based on ultracentrifugal data have been proposed for estimating LDL apoB, but partly owing to their complexity have not been used clinically (11).

In this issue of Clinical Chemistry, Baca and Warnick (12) report a retrospective analysis of nearly 65,000 measurements of plasma triglyceride, total apoB, and LDL apoB performed at the Berkeley Heartlab. After ultracentrifugation at \( d = 1.006 \) kg/L and removal of the supernatant containing chylomicrons and VLDL, apoB (designated LDL apoB) was measured in the infranatant using 3 commercially available reagents each with antibodies to different apoB epitopes on automated chemistry analyzers. Importantly, only samples with triglyceride concentration >2.3 mmol/L (200 mg/dL) were included in the data analysis. After adjusting for triglyceride concentration, a strong positive relationship was observed between total apoB and LDL apoB. The effect of increasing triglyceride concentrations became apparent at a value of approximately 9.0 mmol/L (800 mg/dL). An equation using the plasma triglyceride and total apoB concentrations was used to calculate LDL apoB, and this value was compared with the measured LDL apoB results. Remarkably, a simple linear equation [\( \text{LDL apoB} = \text{total apoB} - 10 - \text{triglyceride}/32 \) (mg/dL) \( \times 2.77 \) [if triglyceride is in mmol/L]] was able to estimate the measured LDL apoB for all 64,895 samples within 12% for each of the 3 methods in 75% of cases.

Ultracentrifugation is a specialist analytical technique that is not readily available in most clinical laboratories. Thus the ability to estimate LDL apoB from measurements of triglyceride and total apoB has merit. The study has some limitations. Samples with a triglyceride concentration <200 mg/dL (<2.3 mmol/L) were not included in the dataset, although there would appear to be no reason why the formula would not apply to normolipidemic individuals. Whether the equation can be universally applied to all lipoprotein phenotypes remains to be determined.

The equation proposed by Baca and Warnick (12) implies that in the absence of hypertriglyceridemia, plasma LDL apoB is approximately 10 mg/dL (0.01 g/L) less than total apoB concentration. However, with increased plasma triglycerides, there is a corresponding and expected increase in the contribution from VLDL apoB. It should be noted that the equation does not refer exclusively to estimation of LDL apoB, since it includes a contribution from LDL apoB and will therefore be limited in patients with dysbeta-lipoproteinemia. A more common source of inaccuracy and imprecision could be attributed to the necessity to measure plasma triglycerides, since concentrations will vary with prandial status and include analytical contributions from both mono- and diglycerides. Interference of plasma triglycerides with the measurement of apoB by immunoturbidimetric assay can be resolved analytically (13). The requirement to measure plasma apoB concentration is consistent with previous literature testifying to its value over LDL cholesterol (5, 6, 9, 10). It is true there are direct methods for estimating LDL apoB that do not involve ultracentrifugation (14), but their precision and accuracy in a routine setting are uncertain. On the other hand, the development of new immunoassays directed at specific epitopes of apoB that reflect small dense or oxidized LDL, and that do not cross-react with apoB-48, may prove more clinically useful and sidestep the need for deriving LDL apoB from 2 separate assays.

The crucial question is whether this equation for LDL apoB will ever be used in clinical practice. This would depend on several factors such as cost, value in CVD risk prediction, clinical context, and contemporary lipid management guidelines. We have estimated that the cost of measuring apoB is fractionally more than the conventional lipid profile (5) but may be more cost effective in CVD risk prediction. Whether LDL apoB is in turn a better predictor of CVD risk than total apoB remains to be demonstrated. Measurement of apoB, or LDL apoB, may be most relevant in patients who are hypertriglyceridemic owing to mixed hyperlipidemia or as an isolated abnormality (5). This encompasses a large number of patients with metabolic syndrome, obesity, type 2 diabetes, chronic kidney disease, and familial hyperbetalipoproteinemia. At least one third of all patients with hypertriglyceridemia have increased apoB, and this initial measurement could be used to ascertain those who despite lifestyle modifications may require statin therapy. LDL apoB could then be applied at a second stage to guide the intensity of statin therapy, but this remains to be demonstrated to be useful and cost-effective. It must be noted that measurement of LDL particle size may be a better end point in individuals treated with fibrates, but these agents should only be used in metabolic syndrome patients after attaining a target concentration of LDL cholesterol, or more importantly apoB, with lifestyle and statin therapy.

Perhaps the greatest impediment to the introduction of apoB, and by implication LDL apoB, is the recommendation of lipid management guidelines from expert bodies. The National Cholesterol Education Program (NCEP) Adult Treatment Panel III guidelines provide targets for LDL cholesterol but not for apoB, let alone LDL apoB, although there is a suggestion that change may be on the horizon (15). By contrast, NCEP does specify that in hypertriglyceridemia \([>150 \text{mg/dL (}>1.7 \text{mmol/L})]\), as in the metabolic syndrome, non-HDL cholesterol concentration should be \(<130 \text{mg/dL}\)
(<3.4 mmol/L) in patients with coronary disease or risk equivalents. In this context, it is noteworthy that in some studies non-HDL cholesterol has been shown to be a better predictor of CVD than total apoB (16). Canadian guidelines, however, specifically recommended a therapeutic target for apoB of <90 mg/dL (<0.9 g/L) in patients with or at high risk of coronary artery disease (17); this will on average correspond to an LDL apoB of <75 mg/dL (<0.75 g/L) in a moderately hypertriglyceridemic patient with type 2 diabetes or metabolic syndrome.

In summary, this new equation for estimating LDL apoB potentially provides additional useful information for managing patients with atherogenic dyslipidemia. However, its clinical value relative to the measurement of total apoB concentration and even non-HDL cholesterol remains to be demonstrated, and then its cost-effectiveness will also need evaluation. Finally, only after expert guidelines adopt apoB as the primary therapeutic target sufficient to produce a paradigm shift in lipid management will the equation have a real chance of being introduced into routine clinical practice.

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