PROGRESS IN
LIPID MEASUREMENT

The Friedewald Formula is challenged by advances in direct LDL measurement

By Gerard Abate, MD, and Daniel Martz

Coronary Heart Disease (CHD) remains the number one cause of death in the United States. Although from 1998 to 2008, the rate of death attributable to CVD decreased by 30.6 percent, more than 220 Americans die each day of CVD, an average of one death every 39 seconds, based on 2008 mortality data. In addition to the consequences of CVD events, the financial burden of CHD treatment on the U.S. healthcare industry cannot be ignored, costing an estimated $297.7 billion in direct and indirect costs.

Risk Factors
The National Cholesterol Education Program's Adult Treatment Panel III identified cigarette smoking, hypertension, low HDL cholesterol, age and a family history of premature CHD as major risk factors exclusive of LDL cholesterol. Additionally, in a review of the NHANES database, Alexander et al noted that NCEP-defined metabolic syndrome affects 44 percent of the U.S. population over 50 years of age, with overt diabetes being 13 percent of this population. Those patients with metabolic syndrome alone had a higher CHD prevalence (13.9 percent). With that said, patients with metabolic syndrome exhibited average LDL cholesterol levels of 144 mg/dL and triglyceride levels averaging 212 mg/dL.

LDL cholesterol treatment targets and guidelines (therapeutic lifestyle intervention versus drug therapy) are determined by the presence of CHD, a CHD comparable risk, or the presence of one or more ATP III-defined major risk factors. ATP/NCEP III guidelines are relatively weak to detect high-risk individuals. Based on ATP-III defined treatment guidelines, many clinicians use a basic lipid panel and Friedewald estimated LDL in conjunction with statin monotherapy to treat CHD risk. However, recent advances in the methods of lipid measurement and growing acceptance of advanced lipid testing are changing the way clinicians provide preventative care to at-risk patients.

Large population-based studies such as the Framingham Heart Study have contributed generalizable knowledge to medical practitioners regarding the effect that individual lipid parameters have on future CHD incidence. Recently, data from the Framingham Offspring Study identified remnant lipoprotein cholesterol as a significant predictor of CHD incidence after 12-year follow-up. HDL cholesterol and HDL subfractions offered significant, independent cardio-protection over the same 12-year follow-up. In addition to these emerging risk factors, these large, population-based studies continue to identify low-density lipoproteins (LDL) as the most significant predictor of cardiovascular disease progression. However, the primary treatment target for preventing CHD incidence and progression, LDL, continues to be estimated in basic lipid panels using the Friedewald Formula.

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**LDL Measurement Challenges**

The basic lipid panel does not measure LDL cholesterol directly, but estimates it using the Friedewald Formula. The Friedewald Formula was developed in the late 1960s as knowledge of the importance and function of lipoprotein subfractions VLDL, HDL and LDL was emerging. To utilize the risk index developed by Gofman and tested in Framingham Heart Study data, a measure of LDL that was more accessible and economical than the Gofman ultracentrifugation process was needed for risk assessment and preventative practice. The Friedewald Formula, which assumes the ratio of triglycerides to cholesterol in VLDL particles is 5, estimates LDL cholesterol with the following formula:

\[
\text{Total Cholesterol} - \text{HDL} - \left(\frac{\text{Triglycerides}}{5}\right) = \text{LDL}
\]

A major limitation of this procedure is that VLDL cholesterol (Triglycerides/5) is calculated under the assumption that VLDL particles have a triglyceride concentration that is five times higher than cholesterol, an assumption which is only true for normal VLDL particles. As the triglyceride concentration in a sample increases, VLDL particles become triglyceride-rich compared with cholesterol, and the Friedewald Formula’s use of the ratio calculating VLDL leads to clinically important underestimation of LDL cholesterol. The Friedewald Formula has other limitations, chief amongst these is the fact that Friedewald estimation of LDL is only possible with serum or plasma samples obtained after a patient fast of 12 hours or greater. Also, LDL estimation cannot be performed if triglycerides are greater than 400 mg/dL and has been shown to be inaccurate when triglyceride values are greater than 250 mg/dL. As well, Friedewald Formula’s limitation in accurately estimating LDL cholesterol at concentrations <70 mg/dL confirmed in “Friedewald-Estimated Versus Directly Measured Low-Density Lipoprotein Cholesterol and Treatment Implications,” published in August 2013 in the *Journal of the American College of Cardiology.*

**Technological Advancements**

Treatment of LDL cholesterol concentration through therapeutic lifestyle intervention and drug therapy remains as important as it was during the development of the Friedewald Formula in the late 1960s. However, technological advancement in the field of advanced lipid testing has come a long way since the days of Gofman’s ultracentrifugation direct measurement. The VAP Lipid Panel (Atherotech Diagnostics Lab), for example, is a density gradient ultracentrifugation technique capable of directly measuring all five major lipoprotein classes: HDL, LDL-Real [LDL minus Lp(a) and IDL components], VLDL, IDL, Lp(a) and their subclasses. The VAP Lipid Panel makes the direct measurement of all five major lipoprotein classes, including direct measurement of LDL cholesterol, an economically and commercially viable method for cholesterol concentration measurement without the need for estimating LDL cholesterol, which is still our most important target of treatment.

The National Cholesterol Education Program’s Adult Treatment Panel III, in addition to setting guidelines for LDL cholesterol treatment, also states that the following emerging risk factors associated with CHD risk are deserving of consideration: Remnant-like particles (RLP), Lipoprotein(a), LDL Pattern, HDL subfractions, Apolipoprotein A-1, and Apolipoprotein B. Many lines of evidence and several smaller studies report a strong association between these emerging lipid risk factors and CHD risk. NCEP guidelines have
not yet fully recommend the routine measurement of all emerging lipid risk factors due to the lack of large, prospective studies confirming what these smaller studies have found—that many of these emerging risks are independent risk factors for CHD incidence with greater predictive power than traditional risk factors. Advances in lipid technology now make it possible for large, prospective population studies to determine their predictive power, independent of other major risk factors. As the role of emerging risk factors in CHD risk is answered, utilization of advanced lipid panels, which provide both directly-measured LDL cholesterol and standardized measures of emerging risk factors, will provide physicians with greater insight into clinically meaningful treatment opportunities.

**Friedewald Estimate vs. Direct Measurement Study**

Martin et al7 performed a first-of-its-kind analysis, “Friedewald-Estimated Versus Directly Measured Low-Density Lipoprotein Cholesterol and Treatment Implications” that compared Friedewald-estimated LDL values with those directly measured via density-gradient ultracentrifugation. Because LDL estimation by the Friedewald Formula is routinely used to guide treatment decisions, researchers sought to determine its compatibility with direct measurement of LDL, a topic that has received very little scrutiny despite previous reports suggesting that Friedewald LDL is underestimated at low LDL cholesterol levels and high triglyceride levels.7

Study patients had a mean age of 59 (SD 15 years of age) and distributed evenly by gender (52 percent female). Lipid distributions closely matched those in the NAHNES Survey. Researchers found that greater differences between directly measured LDL and Friedewald-estimated LDL occurred at lower LDL-C and higher triglyceride levels. Of the 1,340,614 U.S. adults for which VAP directly-measured LDL cholesterol was available, 191,333 patients had Friedewald-estimated LDL <70 mg/dL (excluding 30,174 patients in which triglyceride levels were greater than 400 mg/dL). In these patients, estimated LDL cholesterol was 9 mg/dL and 18.4 mg/dL lower when triglyceride levels were 150 to 199 mg/dL and 200 to 399 mg/dL, respectively.

The Friedewald Formula also proved unreliable at accurately classifying patients at the LDL cholesterol treatment threshold of <70 mg/dL. Twenty-three and 59 percent of patients with estimated LDL <70 mg/dL and triglycerides between 150 to 199 mg/dL or 200 to 399 mg/dL, respectively, had directly-measured LDL cholesterol >70 mg/dL (Figure). The Friedewald Formula fails to accurately estimate LDL cholesterol at clinically important thresholds in the presence of elevated triglyceride levels, especially at low LDL cholesterol levels. These results suggest that, especially in high-risk patients, if triglyceride levels are >150 mg/dL, additional evaluation is necessary to avoid under-treatment.7

These results will have far-reaching impacts on clinical lab testing and guideline development in the near future. Up to 85 percent of American adults reported having undergone lipid testing during a 2009 CDC telephone survey, meaning that clinically meaningful Friedewald Formula underestimation of LDL cholesterol at treatment guidelines of <70 mg/dL has the potential to impact numerous high-risk patients. Inaccuracy and misclassification due to Friedewald underestimation of LDL cholesterol, currently the most important treatment target for CHD risk, especially at clinically important treatment thresholds such as <70 mg/dL—at which it is most important to be accurate—warrants consideration as investigators design future research studies, as experts design new clinical practice guidelines for CHD treatment, and in current patient care.7

Dr. Abate is chief clinical officer, Atherotech Diagnostics Lab; and Daniel Martz is clinical research coordinator, Atherotech Diagnostics Lab.

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