Beyond LDL Cholesterol in Assessing Cardiovascular Risk: apo B or LDL-P?

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Plasma concentrations of LDL cholesterol (LDL-C) are positively associated with increased risk of atherosclerotic cardiovascular disease. There is a variety of robust evidence indicating that this association is causal in nature. First, rare and common genetic variants that specifically influence LDL-C concentrations are also strongly associated with cardiovascular risk (1). Second, interventions that reduce LDL-C, especially but not exclusively statin therapy, reproducibly reduce cardiovascular events (2). In fact, the data with statins are so strong that they are often used in patients whose LDL-C concentrations are not particularly increased, a setting in which statins have still been shown to reduce cardiovascular risk. Thus there is substantial interest in lipoprotein-related biomarkers that provide information about future cardiovascular risk above and beyond LDL-C itself.

LDL contains a core of hydrophobic lipid, mostly cholesteryl ester, a shell of phospholipids, and a single molecule of the large protein apolipoprotein B-100 (apo B). It has long been appreciated that LDL particles vary in size and density, which is largely due to variation in the amount of cholesteryl ester in the particle (3). Because the LDL-C measurement is a measure of the amount of cholesterol being carried in LDL, it is not a reliable measure of LDL particle concentration: small, dense LDL particles have much less cholesterol than large, buoyant LDL particles. Interestingly, however, there is evidence that small, dense LDL may be more atherogenic, which has led to the concept that the overall number of LDL particles may be more predictive of cardiovascular risk than the LDL-C concentration itself (3).

Several methods have emerged that allow a more direct quantification of the number of LDL particles. Because an LDL particle contains a single molecule of apo B, it is possible to directly estimate the number of particles through a simple measurement of apo B concentration (particularly when expressed in molar units). apo B is typically measured by immunonephelometry or immunoturbidimetry, and reagents are available from a wide variety of manufacturers. Standardization of these measurements has been facilitated by the availability of WHO-IFCC reference materials (SP3–07, SP3–08) (4, 5). apo B analytical measurements have shown good reproducibility across laboratories (6%–8% CV in 2012 College of American Pathologists survey), although a number of preanalytical biological confounders, including diurnal and seasonal effects, have been described (6). A number of observational studies have found that apo B concentrations are better predictors of cardiovascular events than LDL-C concentrations (7–9), leading to recommendations by some authorities that apo B be incorporated into guidelines and clinical practice. Indeed, the Canadian Cardiovascular Society incorporated apo B into their 2009 national lipid-management guidelines (10).

A second, independent method to estimate the number of lipoprotein particles in various classes, including LDL, has been developed with nuclear magnetic resonance (NMR) spectroscopy (11). This approach relies on the fact that the NMR signal from the terminal methyl groups of a variety of lipoprotein constituents is proportional to the particle count for a given lipoprotein class. Further, the absolute size of the particle influences the NMR signal: it is possible to take an aggregate measurement and reconstruct the relative proportions of the constituent lipoprotein subclasses. This aggregate measurement allows rapid determination of the spectrum of lipoproteins in an individual, along with a corresponding estimate of their particle concentrations. In particular, this method permits the quantitative estimation of the number of LDL particles (LDL-P). A number of studies have evaluated the relative merits of LDL-P vs LDL-C in predicting cardiovascular events and have generally concluded that LDL-P outperforms LDL-C (12).

Given these 2 independent (and indirect) methods of estimating the number of LDL particles in a given patient, both of which appear to be better than LDL-C...
itself, it is important to ask how they compare in predicting cardiovascular events. There has been uncertainty as to the relative value of these 2 measurement modalities for risk stratification and assessment of treatment efficacy. To address this issue, the AACC Lipoprotein and Vascular Diseases Division Working Group on Best Practices undertook a meta-analysis of 25 existing clinical studies to compare the value of apo B and LDL-P in predicting cardiovascular events. The results of their comprehensive study are presented in this issue of Clinical Chemistry (13).

To assess the relative merits of apo B or LDL-P for risk prediction, the working group identified studies in which these markers were associated with a variety of outcomes, including not any coronary events but also carotid atherosclerosis, diabetes mellitus, and metabolic syndrome. The primary outcome was the detection of a statistical association between outcome and biomarker measurement from one or both of the measurement modalities. On the basis of the presence of statistical association, the working group found robust evidence of an association of apo B and LDL-P with cardiovascular events and concluded that apo B and LDL-P are largely comparable in their association with cardiovascular events. They recommend incorporation of particle number into assessments of cardiovascular disease (CVD) risk. The working group also provided a number of important recommendations for clinical adoption of these assays, including a renewed emphasis on using available resources for standardization, achievement of performance goals, and additional characterization of both measurement modalities. These valuable guidelines will set the stage for further development of lipoprotein particle measurements and their reliable measurement in the research and clinical laboratory.

apo B measurements by immunoassay are compatible with equipment routinely found in the clinical laboratory. In contrast to the variety of assays for apo B, NMR instrumentation for commercial proton measurements of lipoprotein particles has only been available from a single vendor (LipoScience), and NMR spectroscopy has not been widely applied to date for high-volume analysis in many clinical laboratories. Although an NMR analyzer is now approved by the US Food and Drug Administration for use in clinical laboratories, major hurdles such as the large capital investment in an NMR instrument by medical centers must be overcome for more widespread adoption of LDL-P. Primarily on this basis, the working group recommended apo B as the preferred biomarker for development and implementation of guidelines that include the measurement of LDL particle number.

As a practical matter, the working group’s recommendation is understandable given the current widespread availability of apo B assays in clinical laboratories. However, several studies suggest that LDL-P may have a larger role to play and that it would be premature to definitively recommend apo B as the analyte of choice. Although the data show largely overlapping confidence intervals for risk on the basis of apo B vs LDL-P, a number of studies included in the analysis showed at least a modest trend favoring LDL-P with respect to predictive efficacy (Fig. 1 in the working group’s report (13)). As the working group noted, this trend was manifested in both strength of statistical significance as well as in the strength of association (odds ratio, risk ratio) itself. Thus, although both markers may be comparable at the level of bare statistical association with cardiovascular events, LDL-P shows favorable characteristics within a number of the surveyed studies. These characteristics, which extend beyond a dichotomous determination of statistical association, may turn out to be clinically significant. It is, of course, the diagnostic sensitivity and specificity of a biomarker within a selected population rather than the mere presence of a statistical association that is the most critical aspect of performance for patient care. Ultimately, the true clinical value will only be determined by relative behavior of these biomarkers at clinically important cutoff points on their respective ROC curves. Additional work is needed to determine whether the additional assay cost of LDL-P may in fact be offset by a reduction in the cost of care for individuals who demonstrate a false negative risk profile.

Further, although the simple measurements of apo B and LDL-P were found to be comparably associated with CVD risk, it is worth noting that the NMR test reports on a number of other lipoprotein classes, including VLDL and HDL. Two recent reports have suggested that HDL particle number (HDL-P) may be more predictive of cardiovascular risk than HDL-C (14, 15). Furthermore, NMR spectroscopy, which is an inherently multiplexed technology, carries with it the potential ability of simultaneously measuring a wide range of additional analytes, some of which may add further value in cardiovascular risk assessment. This raises the prospect of combining the measurement of a known CVD risk factor (LDL-P) with a more comprehensive metabolomic profiling approach to cardiovascular disease. Although substantial additional work would be required in this area, the NMR approach may have additional future benefits. The ultimate goal of a panel representing the best information on lipoprotein subclasses, their protein and lipid contents, and a variety of other relevant analytes may be best achieved by use of a single measurement (e.g., NMR) rather than a diverse collection of immunoassays.

The aggregate data are remarkably consistent that a measure of LDL particle number, either apo B or
LPL-P, is a better predictor of cardiovascular risk than the classic measurement of LDL-C concentration. A strong case can be made for incorporating a measure of LDL particle concentration into clinical practice when making decisions about initiation or intensification of LDL-lowering therapy. apo B and LPL-P are very comparable in their predictive value, and the decision of which assay to use is largely based on availability, which currently favors apo B. However, if other aspects of the lipoprotein analysis provided by the NMR spectroscopy assay can be convincingly shown to enhance cardiovascular risk prediction, and if its availability increases through placement of instruments in clinical laboratories, it is possible that this approach could eventually emerge as the preferred method of lipoprotein quantification for the assessment of cardiovascular risk.

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References