Are Measurements of LDL Particles Ready for Prime Time?

In this issue of Clinical Chemistry, Ensign et al. (1) report on the differences in 4 methods commonly used to assess LDL subfractions or particles. The results are both interesting and deeply disturbing. In a careful, well-planned study, Ensign and colleagues prospectively and simultaneously collected blood samples as required for each method. Sample analyses by 3 of the methods in question, nuclear magnetic resonance (NMR), density gradient ultracentrifugation (VAP), and gradient gel electrophoresis (GGE), were performed by the laboratories that developed and commercially offer these technologies. The fourth technique, tube gel electrophoresis (TGE), is sold as a commercial reagent set and was performed in the investigators’ own laboratory according to the manufacturer’s specifications.

The primary result investigated was LDL pattern, as interpreted by the reporting laboratories. Other results reported by some of the laboratories included particle number/concentration and LDL cholesterol (LDL-C), the current focus of national and international treatment guidelines and therapeutic goals (2, 3).

Comparison of the LDL pattern results, as interpreted by the reporting laboratories, revealed substantial disparity between the methods, with only 8% (3 of 40) of samples showing complete agreement among the 4 methods for the most basic and qualitative of the interpretations, that of whether the samples analyzed had what is known as pattern A or pattern B. The 2 assays that agreed most often, NMR and GGE, did so for 28 of 37 samples for which results were available for both methods. This comparability was no better than that obtained with the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) III cut-point of 1.70 mmol/L (150 mg/dL) for optimal triglycerides (2), above which pattern B and below which pattern A can be predicted. In the study by Ensign et al, there was observed agreement of NMR with triglyceride results in 28 of 38 samples for which results were available. In the discrepant samples, 5 classified as pattern B had borderline triglyceride concentrations of 1.55 to 1.64 mmol/L (137–145 mg/dL), and of those, most were reported as pattern A by other methods. Furthermore these samples with low triglycerides had LDL-C concentrations of 1.5 to 2.25 mmol/L (58–87 ng/dL), a concentration interval not associated with increased atherosclerosis risk or requiring intervention according to any guidelines. This triglyceride cut-point was also closely associated with GGE results (23 of 35 or 28 of 35 when pattern A/B was reported). Thus, this study suggests that triglyceride measurement is as useful for predicting LDL pattern as any of the far more elaborate and expensive technologies.

When particle size was compared, substantial differences and poor correlations were seen for NMR, GGE, and TGE. Given that the particles separated by NMR are not necessarily the same as those separated by density or electrical charge, comparability of results among these assays is unlikely to improve regardless of what standardization or common calibration is attempted.

Perhaps the most disturbing findings reported by Ensign et al., given the consequences for current clinical practice, which is driven by NCEP ATP III guidelines (2, 3), were the substantial differences in results for LDL-C concentrations. Mean concentrations obtained with the 3 methods ranged from ~3.37 mmol/L (130 mg/dL) for TGE to 4.66 mmol/L (180 mg/dL) for NMR, with density gradient ultracentrifugation falling somewhere in between. Thus, this study raises serious questions about the reliability of clinical decisions and treatment choices for clinicians using these technologies or laboratories and relying on both the LDL pattern and the LDL-C concentration. Better agreement in LDL-C between laboratories would have been expected, and with better standardization, such as a common calibrator traceable to CDC, the discrepancies would likely diminish markedly (4).

In addition to the unreliability of LDL pattern testing documented by Ensign et al. (1), the conflicting clinical evidence regarding the usefulness of LDL patterns and particle size raises questions regarding their use for guiding diagnosis and treatment (5). The evidence of clinical benefit of LDL reduction, from more than 20 event-based trials involving more than 100,000 patients, has become indisputable (6). For this reason LDL-C reduction is now the cornerstone for prevention of coronary artery disease (CAD) (2). Because additional evidence from controlled clinical trials becomes available, the concentrations of LDL-C at which intervention is started and the goals for optimal therapy are being constantly revised downward (3). LDL-C measurement is a standard tool for guiding intervention and treatment and is such a reliably demonstrated risk factor that it might be thought of as a causative agent for CAD, but it is by no means perfect. LDL-C measurement can be subject to inaccurate measurement in many laboratories, especially where so-called direct or homogeneous assays are used, and it may lead to under- or overestimation of CAD risk.

Because of the high prevalence of CAD in our society, attempts have been made to find risk factors with greater clinical specificity and detection limit. An area of research for nearly 3 decades has been the subfractions of circulating lipoproteins, mainly those in the LDL class (7). The key to understanding these particles relates to the metabolism of the apolipoprotein B100 (Apo B)-containing particles secreted by the liver. Apo B is released mostly as large, lipid-enriched VLDL particles, which are remodeled as they circulate through the body until they are eventually removed from the blood stream as cholesterol-poor LDL via the Apo B/LDL receptor. Increased production rate, disruption of remodeling, or decreased clearance rate of any of these Apo B–containing lipoproteins may lead to their accumulation in the circulation, oxidation and uptake into the arterial wall, initiating or aggravating an inflammatory process that leads to atherosclerosis and CAD. Thus all Apo B–containing lipoproteins can be considered atherogenic (8).

Apo B has long been advocated as a replacement for, or adjunct to, LDL-C, especially when triglycerides are in-
increased. In 2001, at the time of NCEP-ATP III, measurement of Apo B was not yet sufficiently standardized, widely available, or well understood by clinicians to advocate its use. Non-HDL-C was suggested by the ATP III as a secondary target after LDL-C (2), as an attempt to capture additional Apo B–containing particles, especially those in the small VLDL and LDL range that are also considered to be atherogenic (7). In the last 5 years, however, these technical and availability problems with Apo B measurement have been overcome, and several pivotal studies, both epidemiologic (9,10) and interventional (11) have further demonstrated the clear advantage of Apo B over other lipids.

In the last decade, techniques for assessing LDL subclasses have become faster and less expensive, and high-throughput techniques as outlined by Ensign et al. (1) have been developed and incorporated into routine clinical practice (12), a process driven mainly by commercialization and marketing.

With all of this in mind, what does LDL subclass analysis by any of the available techniques offer? Certainly in a purely research mode, LDL subclass analysis may be helpful for understanding minor modulations in the Apo B cascade related to various disorders such as diabetes or insulin resistance, or for assessing the mechanism of new drugs.

For the practicing clinician, however, the major argument for extending measurement of subclasses into the mass market is the hypothesis that one subclass is more atherogenic than another. Because evidence clearly indicates that all Apo B–containing particles are atherogenic (8), this reasoning is akin to the argument that an Uzi submachine gun is more deadly than an M16 or an AK47. Obviously all are potentially lethal, and although this assertion may interest gun aficionados, it matters little to law enforcement or to general public safety if the sole objective is disarmament! As has been demonstrated in clinical trials, if Apo B, or non-HDL-C or even LDL-C is decreased sufficiently, there will be a reduction of all types of particles, even if some are decreased slightly more than others, with a resulting beneficial impact on atherosclerosis and CAD. To suggest that shifting the focus from one particle to another without impacting the total circulating Apo B burden or reducing it will decrease CAD risk is, to use the gun analogy, like believing public safety will improve by switching gun users with Uzis to AK47s. In LDL subclass parlance, this method is known as changing those with pattern B to pattern A, or showing that when one subclass is decreased a little more than another the result is a relative increase in the percentage of the remaining denser particles. To be evenhanded to those advocating subclasses, it is important to examine the evidence for the claims. Recent reviews of evidence for these claims, from biological, epidemiologic, and clinical trials (4,8), have found no strong substantiation that any particles in the VLDL, IDL, or LDL range are not atherogenic or that some are more atherogenic than others. Subclass studies have proliferated over the last few years, but many of these studies were funded or subsidized either by suppliers of the assays as a method to expand their use and move them into mainstream practice, or by pharmaceutical companies in an attempt to claim some advantage over other therapeutic agents, especially when the LDL-C or Apo B reducing ability of their drug was less competitive. Although these studies have created more heat, they provide little additional light.

Thus, current data on LDL subclasses are at best incomplete and at worst misleading, suffering from publication bias, and now, given the results of the Ensign et al. study, unreliable. A reasonable conclusion is that, although assessing LDL and HDL subclasses may be of academic and research interest, the solid evidence for their use in routine clinical practice is lacking, and the available evidence is conflicting and confusing. In addition, given that determination of LDL particle number may well be a more valid approach, Apo B measurement offers an easier, more reliable, more standardized, and less expensive measurement while providing clinicians with a simpler underlying concept for understanding and treatment targets already defined in some current guidelines.

References


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