To apoB or not to apoB: new arguments, but basis for widespread implementation remains elusive

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How best should we measure circulating lipoproteins to facilitate the prevention of atherosclerotic cardiovascular disease (ASCVD)? Cholesterol was first identified in human blood in 1833 by Félix-Henri Boudet (1), and in 1913 Anitschkow showed cholesterol caused atherosclerosis in rabbits. (2) Interest in LDL-cholesterol (LDLc) *per se* began in 1955, when Gofman used ultracentrifugation to separate cholesterol-carrying lipoproteins in plasma according to density, identifying low and high density (LDLc and HDLc) fractions (3). The idea that not all lipoproteins were the same in terms of ASCVD transformed our understanding of atherosclerosis.

Over time technological advances have driven ever expanding options for measuring different lipid fractions or lipoproteins, including by physical properties (such as ultracentrifugation techniques, gel electrophoresis, nuclear magnetic resonance) and biochemical characteristics (total cholesterol, HDLc, and direct LDLc). Advances in understanding have also led to multiple options for calculating different fractions including non-HDLc (total cholesterol minus HDLc), or LDLc (Friedewald, Sampson, & Martin/Hopkins equations). Adding to this complex mix, apolipoprotein B (apoB) immunoassays have been around since the 1970s (4) but to date, have been little used in clinical practice. As such, our definition of what is the best measure of "bad" cholesterol to measure lipid-associated ASCVD risk continues to be debated.

Clinical guidelines have tended to focus on measurement of LDLc and non-HDLc (5). However, apoB is recognized as a marker of lipoprotein function and particle number; rather than just reflecting cholesterol content, apoB measurement captures all of the lipoproteins causal in atherogenesis (6). Therefore, why not just measure apoB instead of approximating risk with less accurate surrogates such as cholesterol levels?

In that vein of reasoning, articles by Cole et al (7) and Pencina et al (8) build on an established literature (6), advocating that apoB should be routinely measured in patients. Pencina et al use data from three different cohort studies and show that apoB is consistently associated with risk of coronary heart disease (coronary death or nonfatal myocardial infarction), but LDL-c/apoB ratio (a putative surrogate of the cholesterol content of lipoproteins) is not. Cole et al took a different approach and, using a clinically relevant group of sequential patients, developed an equation for "predicted LDLc" based on measuring apoB. They report that among individuals with very low measured LDLc, 40% had discordantly higher "predicted LDLc" than measured LDLc, inferring that that LDLc measurement is potentially misleading. They argue that this discordance will lead to

misclassification of ASCVD risk, and therefore propose apoB equivalent units as targets in clinical guidelines.

Whilst both studies are informative and are broadly well conducted, in our view they underemphasise potentially informative data. First, Pencina et al prominently report in the abstract a correlation between apoB and LDL-c of r>0.80, a result suggesting the biomarkers are not interchangeable. However, in UK Biobank, the most contemporary and by far the largest of the three cohort studies (more than 10 times larger than the Women's Health Study, and 100 times larger than Framingham study), the correlation of apoB with LDLc and non-HDLc was r=0.96 (i.e. nearly interchangeable). As such, UK Biobank data suggests ~92% of the variability in apoB is "captured" by measuring LDLc or non-HDLc. These data very much fit with our own analyses of UK Biobank, where we saw similar correlations and also showed that apoB and apoA1 measurements do not add to conventional ASCVD risk scores once LDLc or non-HDLc are already included (9). This conclusion is further supported by previous meta-analysis of cohort studies (10). Indeed, if table 3 had reported LDLc and non-HDLc without adjustment for apoB, the strength of their associations with CHD, would, we believe, be very similar to that seen for apoB alone (9).

Secondly, in the study by Cole et al they use a variety of approaches to define discordance, some of which involve potentially small discrepancies (e.g. a patient with measured LDLc between 60-70mg/dl would be discrepant if their predicted LDLc was 71mg/dl); such an approach should be placed in context. In intermediate precision assays (multiple tests of the same sample) conducted by the assay manufacturer, the standard deviation of the apoB assay was 3mg/dl (stated coefficient of variation 3.1%) at an apoB concentration of 83mg/dl (11). As such, if a patient sample with a true apoB concentration of 83mg/dl is measured multiple times, 95% of the time we expect the assay to return an apoB result in the 77mg/dl-89mg/dl range. This would in turn lead to "predicted LDLc" from the equation in the range 77-94mg/dl. This simple illustration overlooks the impact of additional error in measurement in LDLc within the model. Therefore, a proportion of the observed 'discordance' between predicted and measured LDLc in Cole et al's study could simply be due to assay noise. Even so, there clearly are some patients with more substantial discordance between observed and predicted apoB; for instance, 16.7% of patients had predicted LDLc at least 20mg/dl higher or lower than measured LDLc, although whether such differences are important for ASCVD risk estimation is less clear.

Thirdly, the issue of discordance was examined in our previous analysis of UK Biobank data where absolute difference of >10% between apoB and LDLc percentiles was used to stratify the population into a discordant group. (9) By this definition, ~18% of participants had discordant LDLc and apoB measurements, and apoB was more strongly associated with ASCVD risk in those participants, in agreement with other data (12). Ultimately however, *even* in discordant participants measurement of apoB and apoA1 did *not* change the C-statistic / area under the receiver operating characteristic (ROC) curve of ASCVD risk scores when added to usual classical risk factors (+0.0007; 95% CI −0.0011, 0.0024) (9). This lack of improvement in discrimination is in part likely because other risk factors measured in ASCVD risk scores compensate for apparent discordance. In the Framingham study, discordant apoB and LDLc are associated strongly with age, sex, diabetes, hypertension, and smoking (13); yet, all of these factors are already included in most ASCVD risk calculators. The c-statistic is only one measure of clinical utility, and has some limitations, but the onus is very much on proponents of apoB to show that its measurement meaningfully changes clinical decisions when set against currently used ASCVD risk scores.

Fourthly, all of the above discussions bring us back to the question as to whether moderate discordance matters in clinical care. For those concerned about whether the risk factors we measure are directly causal in ASCVD, there may be an argument for measuring apoB more widely. If, however, we look objectively at the clinical framework we operate in, lipids are rarely measured in isolation and a much wider panel of risk factors is considered before clinicians target ASCVD prevention. Many risk factors in ASCVD risks score are subject to misclassification to one degree or another (for example, smoking status relying on patient history rather than cotinine measurement, or office measurements of blood pressure rather than 24-hour ambulatory measurements). That is not to say misclassification should be actively encouraged, but that the benefits of these commonly used “surrogates” such as patient history in the case of smoking status are that they are practical, cheap, easily understood, and highly correlated with what we want to measure. Could we theoretically measure more accurate or causal risk markers? Yes, absolutely - but we must ask if healthcare authorities are willing to pay for that investment. Clearly, different authorities in different settings will use different metrics to make that decision. The United States Centers for Medicare and Medicaid Services (CMS) prices a standard lipid panel test at $13.39 and a lipid panel plus apoB test at $34.48 (14). An extra $21 may not seem like a lot, but multiplied by millions of patients, it appears to us an unnecessary expense and burden if it rarely changes treatment decisions for the better.

Finally, some may argue that apoB measurement may be the best metric to treat to target once a patient is on cholesterol lowering treatments. However, a report from the joint consensus panel of the European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) reported insufficient evidence to support apoB measurement to replace standard lipid profiles. Rather, they suggested that simple non-HDLc could supplement LDLc as an additional target test (5).

No doubt the arguments for using apoB will continue to be made, but examining this issue from many angles suggests traditional lipids tests are cheap, pragmatic and effective. More data, including assessment of cost effectiveness and feasibility, are likely to be required to change an already efficient formula for targeting and monitoring lipid lowering interventions.

Conflict of Interest Statement

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