Cost-effective biomarker for coronary artery disease: time to move on

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Serum cholesterol, and in particular LDL cholesterol levels, has been the well-entrenched principle proatherogenic lipoprotein-related marker for the risk of vascular diseases. However, our increasing insights into the pathophysiology of cardiovascular diseases and also data from multiple well-conducted epidemiological studies and clinical trials have distinctly demonstrated the superiority of apoB along with apoB/apoA-1 ratio over conventional measurements of lipids. This superiority of apoB has been proven in population-based studies and also in specific high-risk groups like patients on lipid-lowering therapy, diabetics and South Asians. In this article, we review the current available evidence to demonstrate why apoB and the apoB/apoA-1 ratio should now be the preferred biomarker of coronary artery disease.

Keywords: Apolipoproteins, coronary artery disease, cost-effective biomarker, serum cholesterol.

CARDIOVASCULAR diseases (CVD) have emerged as the leading cause of morbidity and mortality worldwide[1]. To date, serum cholesterol, and in particular LDL cholesterol levels, has been the principle proatherogenic lipoprotein-related marker for the risk of vascular diseases. LDL cholesterol has also been the principal target of hypolipidemic therapy. However, based on a large indisputable body of evidence, consensus statements issued by the American Diabetes Association (ADA) and the American College of Cardiology (ACC) have called for a major change. They have concluded that apolipoprotein B (apoB) is more accurate than either LDL cholesterol or non-HDL C cholesterol in determining the adequacy of LDL-lowering therapy. This conclusion is based on the advances in our understanding of the pathophysiology of CVD as well as data from multiple well-conducted epidemiological studies and clinical trials that have demonstrated the superiority of apoB along with the apoB/apoA-1 ratio over conventional measurements of lipids. We review this evidence herein and discuss why apolipoproteins and not the cholesterol content in LDL and HDL

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should be the lipoprotein-related biomarkers of choice in CVD.

Why choose apoB over LDL cholesterol?

Pathophysiological reasons

Notwithstanding that LDL cholesterol is presently the standard of care for lipid management, the approach has severe limitations. First, there is considerable variance between the value determined in the clinical laboratory, whether by calculation or by direct measurement and the actual value as determined by beta-quantitation. This error is overcome in epidemiological studies or clinical trials when the trends of large numbers are determined. It is uncorrected – and the errors that follow not appreciated – in clinical practice when decisions are based on single samples. Second, because LDL particles differ in composition, with some containing more cholesterol in their core and others less, and because small, dense, cholesterol-depleted LDL particles are so common in patients with vascular disease, LDL cholesterol frequently underestimates LDL particle number. It is the entry and trapping of LDL particles within the arterial wall that cause atherosclerosis and evidence now indicates all LDL particles – those that are larger and contain more cholesterol as well as those that are smaller with much less cholesterol – are equally atherogenic. The likelihood that an LDL particle will enter the arterial wall and initiate and/or sustain the atherosclerotic process is directly related to the number of LDL particles within the lumen of the artery and not to the total mass of cholesterol within it.

ApoB measures total atherogenic particle burden because each atherogenic particle – that is to say, each VLDL, IDL, LDL and Lp(a) particle – contains one molecule of apoB100. In addition, in individuals with small, dense cholesterol-depleted LDL, LDL cholesterol will necessarily underestimate LDL particle number. LDL cholesterol may be normal as, for example, it typically is in patients with diabetes, insulin resistance, abdominal obesity or premature coronary disease, but apoB is characteristically high. Indeed, the typical lipid profile for all these groups is that of a normal or near-

normal LDL cholesterol, low to normal HDL cholesterol, raised triglycerides and high apoB levels, that is, hyperTg hyperapoB[1,2].

Epidemiological evidence

Several large, prospective investigations have now studied LDL cholesterol and apoB as predictors of cardiovascular risk. The evidence from these studies is summarized here.

The Apolipoprotein-related Mortality Risk Study (AMORIS)[3] was designed to compare concentrations of LDL cholesterol and apoB as predictors of fatal acute myocardial infarction (MI) in 175,553 adults followed up for 5.5 years. ApoB and apoA-1 were highly significant predictors at any concentration of total cholesterol and triglyceride in both sexes and at all ages. LDL cholesterol concentration was marginally significant in men, but not in women, and not in older patients. The strongest univariate predictor was the apoB/apoA-1 ratio. Moreover, in multivariate analyses, this ratio was better than any cholesterol ratio in both men and women at the prediction of cardiovascular risk. Receiver Operating Characteristics technique demonstrated higher sensitivity and specificity of apoB over LDL cholesterol in predicting future cardiovascular risk.

The Quebec Cardiovascular Study, a 5-year cohort study of 2155 men, reported similar findings[4]. In stepwise multivariate analyses, concentration of apoB showed the strongest association with risk of coronary events.

Another post-MI study, the THROMBO study[5] investigated the predictive role of six haemostatic and seven lipid factors on risk of recurrent coronary events in 1045 patients followed up for 4 years. ApoB was significantly associated with increased coronary event rates in univariate analysis, whereas LDL cholesterol was not. By multivariate analysis, only the amount of apoB, apoA-1 and the D-dimer of fibrinogen remained the significant predictor.

The Northwick Park Heart Study[6], another prospective study over a 6-year period in 2508 middle-aged men free of coronary disease at baseline, proved the superiority of lipoproteins. The study demonstrated that apoB predicted risk better than total or LDL cholesterol, the association of hypertriglyceridaemia with apoB increased risk, the pair of triglyceride and apoB was superior to the pair of triglyceride and cholesterol, and finally, the ratio of apoB/apoA-1 had the strongest effect on risk.

The EPIC-Norflok study[7], a nested case control study, studied the risk of future coronary events. Again, apoB/apoA-1 was the strongest predictor of risk and remained significantly discriminatory in multivariable analysis, while total/HDL cholesterol lost its significance. ApoB/apoA-1 ratio also remained significant in an analysis that adjusted for the Framingham risk score. Despite its stronger predictive power, the apoB/apoA-1 ratio did not add to the area under the curve in ROC analysis vis-a-vis total/HDL cholesterol ratio, though it was superior to the Framingham risk stratification score.

Similar data have emerged from two recently published, prospective, cohort studies from two vastly different populations, one Chinese and the other Danish, that again confirmed apoB as the strongest predictor of future cardiovascular event[8,9]. Thus the results of these large, well-conducted studies show that apoB is superior to total cholesterol or LDL cholesterol to predict the risk of vascular disease, and the ratio of apoB/apoA-1 is the strongest predictor of overall index of risk. ROC analysis is less sensitive than hazard ratios to discriminate between risk factors.

Evidence from on-therapy group in clinical trials

AFCAPS/TexCAPS was a primary prevention trial of lovastatin[10]. Based on their 1-year on-treatment concentrations, none of the on-therapy lipid parameters – including LDL cholesterol, non-HDL cholesterol and total/HDL cholesterol ratio – were significant predictors of clinical events. On the contrary, both apoB and apoB/apoA-1 ratio on treatment predicted future cardiac events. The slopes relating on-treatment values of apoB and risk for patients in control and treated groups were almost identical, suggesting a continuous relation between apoB and risk of events.

Similarly, the LIPID trial[11] showed that while on-treatment apoB levels were predictive of risk, LDL cholesterol levels were not. It also revealed that apoB/apoA-1 ratio was a stronger predictor of risk than total/HDL cholesterol.

Evidence from the Leiden Heart Study[12], a secondary prevention trial, was similar. Multivariate analysis revealed that on-treatment apoA-1 and apoB were the only significant predictors for future cardiovascular events. On-treatment levels of total and LDL cholesterol, and triglycerides were not associated with increased risk of recurrent cardiovascular events in CAD patients treated to target levels. However, on-treatment levels of apoB and in particular apoA-1 were significantly predictive for MI and all-cause mortality.

Besides this superiority of apoB over LDL cholesterol was also seen in CARE and FATS trial[13,14] and in the fibrate trials: DIAS[15] and the BECAIT[16]. Thus evidence from lipid-lowering trial clearly suggests that though LDL cholesterol loses predictive power for further risk stratification in patients on therapy, apolipoproteins maintain their discriminatory value. These data form the bases for the consensus statements by the ADA and the ACC that apoB is the best test of the adequacy of LDL-lowering therapy[17].

Superiority in diabetics

Diabetics are at high risk for cardiovascular diseases and diabetes is considered to be a 'coronary heart disease (CHD) equivalent'[18]. Translated, this means that the risk for a cardiovascular event in diabetics is as high as that in an individual with established CHD. The reasons for this are not entirely well understood. Improvement in blood sugar control has not been shown to reduce cardiovascular events significantly[19]. One reason may be the critical role that atherogenic dyslipoproteinaemia, hyperTg hyperapoB, plays in these patients. The levels of total and LDL cholesterol are characteristically normal in these patients and therefore do not explain the increased risk[20]. On the other hand, triglycerides do tend to be elevated and HDL cholesterol tends to be low. More importantly, when analysed for apolipoproteins, nearly half of the normocholesterolemic population had raised apoB levels[20]. Thus, given its independent association with CVD and that it identifies high-risk phenotypes in normocholesterolemic diabetic patients, apoB should be used to evaluate the lipoprotein-related risk of vascular disease in these patients.

Estimation methodology

For any biomarker to have widespread use in clinical management, it is mandatory that there be a rigorously standardized, accurate, automated and affordable method to measure it. Concern has been raised earlier regarding the measurement of apoB; however, current methods are well standardized, precise and inexpensive, and have been extensively used in epidemiological studies. Paradoxically, it is becoming increasingly obvious that the methodological weaknesses lie with calculated LDL cholesterol. First, measurement of LDL cholesterol requires blood sampling in a fasting state, unlike the apoB measurement which can be performed in fed state. Secondly, while it is well known that LDL cholesterol cannot be calculated if plasma triglycerides are >400 mg/dl, it is not generally appreciated that the calculated LDL cholesterol levels may be biased and erroneous at lower levels[21] or even at normal values of triglycerides[22]. Tighe et al.[21] reported that concordant results for NCEP ATP-III risk categories were present for only 48.1% of samples. Similarly, calculated LDL cholesterol has been reported to be fallacious in diabetics[23], nephrotic syndrome[24] and liver disease[25]. Third and probably the most crucial lapse in LDL cholesterol measurement occurs at low values of LDL cholesterol, below 120 mg/dl, the current target of desired lipid levels. Scharnagl et al.[26] compared LDL cholesterol calculated by the Friedewald formula with the values determined by beta quantitation, the standard reference method. They found that though there was good correlation over a wide range of values, substantial differences occurred at LDL cholesterol levels < 120 mg/dl between the two methods. Thus the Friedewald formula tends to be unreliable at desired therapeutic levels of LDL cholesterol, and obviously this may adversely impact patient management. Though direct methods for assessment of LDL cholesterol are present, they are expensive and not widely available and not well standardized.

Why apoB and not non-HDL cholesterol

Non-HDL cholesterol has been proposed as an alternative predictor to LDL cholesterol in patients with hypertriglyceridaemia[18]. As there is a good correlation between non-HDL cholesterol and apoB, measurement of non-HDL cholesterol is considered to be a substitute for measurement of apoB[18]. However, this correlation is only an approximate index of agreement between two variables. There can be a high correlation coefficient despite considerable dispersion above and below the line of identity. This holds true for non-HDL cholesterol and apoB.

This is well borne out from clinical trials in which on-treatment concentration of apoB was superior to non-HDL-cholesterol in the Leiden Heart Study[12] and in AFCAPS/TexCAPS[10]. Another cohort study, Health Professionals Follow-up Study, showed that though non-HDL-C and apoB were both strong predictors of CHD on univariate analysis, when non-HDL-C and apoB were mutually adjusted, only apoB was predictive of CHD[27]. Similarly, apoB was better than non-HDL cholesterol to predict carotid intima media thickness in patients with familial combined hyperlipidaemia, which is the commonest familial atherogenic dyslipoproteinaemia and is characterized by hypertriglyceridaemia and raised concentration of apoB[28]. Thus in view of these data, the argument that non-HDL-cholesterol can serve as a surrogate for apoB seems flawed.

South Asians

South Asians are a high-risk group for developing CHD. Studies in migrant Indians and cross-country comparisons in registries have demonstrated that South Asians have much higher mortality and case fatality rates due to CHD compared to other ethnicities[29-31]. This has led to the speculation that hitherto unidentified non-traditional risk factors may be responsible for their susceptibility to CHD. However, the INTER-HEART study[32] revealed that attributable risk due to conventional risk factors among South Asians was similar to all other populations world wide. Further, the apoB/apoA ratio accounted for 47% of the population-attributable risk of incident MI in South Asians. Although this study did not compare the apoB/apoA to the total/HDL cholesterol or LDL/HDL cholesterol ratio, there are reasons to believe that the former maybe a better biomarker in South Asians. Most

of the evidence to this effect is circumstantial or indirect, as there is currently no study comparing these two ratios head to head. For example, a similar, well-designed case-control study in patients of MI from South India failed to show lipids as a risk factor[33]. In this study cholesterol fractions and not apolipoproteins were used as determinants of dyslipidaemia prevalence in MI patients and controls. Another important reason to believe this notion is the high incidence of diabetes and impaired glucose tolerance among South Asians. A study from Canada revealed that the glucose intolerance (diabetes and impaired glucose tolerance) is prevalent in one-third of the population in South Asians[34]. A similar high rate of 15–20% prevalence of diabetes is reported among migrant South Asians in studies from the UK and USA[35,36]. Prevalence of metabolic syndrome is also high in South Asians, with reported rates of 34% in the urban Indian population and 12% in rural Indians[37]. Similarly, prevalence of smaller and denser LDL particles is reported to be higher in South Asians[38].

As discussed earlier in this article, individuals with diabetes, metabolic syndrome and small dense LDL cholesterol are the ones where apolipoproteins have proven to be superior to cholesterol fractions in assessing CVD risk. Thus logically it seems prudent to conclude that apolipoproteins may be a superior biomarker of risk for CVD in South Asians.

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