The Role of Advanced Lipid Testing in Clinical Practice

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The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines recommend assessing an individual’s cardiovascular (CV) risk from the Framingham risk score; however, the Framingham risk score may underestimate coronary heart disease (CHD) risk. Current guidelines have identified some emerging lipid risk factors that can be measured by several commercially available advanced cholesterol tests. These emerging lipid risk factors are meant to supplement the Framingham risk score to help the clinician to better assess CV risk.

Although advanced lipid testing cannot be recommended for routine screening, it may be of value in individuals with a family history of premature CHD, postmenopausal women, and individuals at intermediate risk for CHD, especially if they are near the boundary of being at high risk. This review examines the role of advanced lipid testing in clinical practice.

To reduce the risk of CV disease, the leading cause of death in the United States, physicians have typically focused on modifying established CV risk factors. Hyperlipidemia is one of these factors, and lipid-lowering therapy has emerged as an important means to prevent CV events.

The NCEP ATP III guidelines recommend lipid-lowering therapy based on an individual’s CV risk, with low-density lipoprotein (LDL) cholesterol as the primary target of lipid-lowering therapy. Based on these guidelines, individuals with CHD, diabetes mellitus, or vascular disease are considered high-risk and have an LDL cholesterol goal of <100 mg/dL with an optional goal of <70 mg/dL. From the number of traditional risk factors present, the risk of an individual without vascular disease or diabetes is calculated with the Framingham risk score. Individuals whose 10-year risk of a CV event is between 10% and 20% are categorized as being at moderate risk and their LDL cholesterol goal is set at <130 mg/dL with an optional goal of <100 mg/dL. The LDL cholesterol goal for low-risk individuals with a 10-year CV risk <10% is <160 mg/dL.

The Framingham risk score may underestimate risk, especially in women and individuals at risk for premature CHD. This is because patients at risk for CHD may lack the traditional risk factors used in calculating their Framingham risk score. In an analysis of 14 large clinical trials in CHD, only 38% of patients had ≥2 major risk factors present. Many of these patients would not have been considered high-risk based solely on their Framingham risk score. Akosah and colleagues identified 222 younger patients (men younger than 55 years and women younger than 65 years) who were hospitalized for acute myocardial infarction. Their mean lipid levels were normal, and only 25% of all patients and 18% of women would have met the criteria to start lipid-lowering therapy based on their Framingham risk score.

The NCEP ATP III guidelines have identified several emerging risk factors that can be used in clinical practice. These lipid-related and non-lipid-related emerging risk factors are meant to supplement the Framingham risk score to help the clinician to better assess CV risk. Several advanced cholesterol tests are now commercially available that can measure the emerging lipid-related risk factors and thus have a role in clinical practice.

ADVANCED CHOLESTEROL TESTING

The standard cholesterol test measures total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol levels and provides an estimate of LDL cholesterol. We now recognize that each of these lipoproteins is made up of subpopulations that vary in size and density (Figure 1). Three commercial laboratories provide advanced lipoprotein testing to measure these different lipoproteins and their subclasses (Table).

The Vertical Auto Profile (VAP) Cholesterol Test (Atherotech, Birmingham, AL) isolates lipoproteins...
by a single, vertical spin, density-gradient ultracentrifugation method. The analysis by VAP directly measures LDL cholesterol, intermediate-density lipoprotein (IDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol, lipoprotein(a) (Lp(a)), and HDL cholesterol concentrations. In addition, the subclasses of LDL, VLDL, and HDL are measured and reported. LipoScience (Raleigh, NC) markets an advanced lipoprotein test that uses nuclear magnetic resonance (NMR) technology to quantify LDL, VLDL, and HDL cholesterol subclasses. The NMR device measures LDL particle number, although this number can be estimated by other means because it is a reflection of LDL particle size and LDL cholesterol concentration. Because Lp(a) and LDL are similar in size, these 2 entities cannot be separated by the NMR analysis; therefore, Lp(a) is not measured by this technology. The third commercially available advanced lipoprotein test is from Berkeley Heart Labs (Burlingame, CA) and uses segmented gradient gel electrophoresis technology to measure LDL peak particle diameter as well as the concentration of the major LDL cholesterol subclasses. This same technology can be used to separate HDL into its unique subclasses. Total LDL cholesterol, HDL cholesterol, Lp(a), and other lipid parameters are also measured by Berkeley Heart Labs.

In the standard cholesterol test, LDL cholesterol is estimated from the Friedewald equation \((\text{LDL cholesterol}) = (\text{total cholesterol}) - (\text{HDL cholesterol}) - (\text{triglycerides}/5)\). Directly measuring LDL cholesterol concentrations is more reliable under certain conditions. LDL cholesterol concentrations will be underestimated by the Friedewald equation in patients with hypertriglyceridemia, obesity, or diabetes. In fact, most laboratories will not report a calculated LDL cholesterol level when triglyceride levels are >400 mg/dL. In addition, when LDL cholesterol levels are low, calculated LDL cholesterol levels will underestimate actual concentrations by up to 20%. A further advantage of directly measuring LDL cholesterol is that patients do not need to be fasting.

**EMERGING LIPID-RELATED RISK FACTORS MEASURED BY ADVANCED CHOLESTEROL TESTING**

**Small and Dense LDL Cholesterol**

Within each individual exist LDL particles of varying size, density, and lipoprotein composition. For example, at any given concentration of LDL cholesterol,
thought to be the most atherogenic LDL subclass.\(^7\)

Small and dense LDL particles are more likely to enter the arterial wall and be oxidized, and they are taken up and degraded by the kidney, lowering the concentration of HDL cholesterol (Figure 2). HDL cholesterol, such as patients with diabetes mellitus or the metabolic syndrome. The production of small and dense LDL particles is linked metabolically to an overproduction of large VLDL particles. Triglycerides in large VLDL particles are exchanged for cholesteryl esters on large LDL particles by cholesteryl ester transfer protein (CETP), enriching these LDL particles with triglycerides. The triglycerides in LDL particles are hydrolyzed by hepatic lipase, producing a small and dense cholesteryl ester–depleted LDL particle.\(^7\)

In a similar fashion, HDL particles become triglyceride-enriched by exchange of cholesteryl esters for triglycerides by CETP. Hydrolysis of triglycerides in the HDL core by hepatic lipase produces smaller HDL particles that are more avidly taken up and degraded by the kidney, lowering the concentration of HDL cholesterol (Figure 2).

Small and dense LDL particles are more likely to enter the arterial wall and be oxidized, and they are thought to be the most atherogenic LDL subclass.\(^7\) Individuals whose LDL is predominately small and dense would be at higher atherogenic risk than an individual whose LDL particles are predominately large and buoyant, even if both have similar concentrations of LDL cholesterol. Small and dense LDL is often present in patients with elevated triglyceride levels and low levels of HDL cholesterol, such as patients with diabetes mellitus or the metabolic syndrome. The production of small and dense LDL particles is linked metabolically to an overproduction of large VLDL particles. Triglycerides in large VLDL particles are exchanged for cholesteryl esters on large LDL particles by cholesteryl ester transfer protein (CETP), enriching these LDL particles with triglycerides. The triglycerides in LDL particles are hydrolyzed by hepatic lipase, producing a small and dense cholesteryl ester–depleted LDL particle.\(^7\) In a similar fashion, HDL particles become triglyceride-enriched by exchange of cholesteryl esters for triglycerides by CETP. Hydrolysis of triglycerides in the HDL core by hepatic lipase produces smaller HDL particles that are more avidly taken up and degraded by the kidney, lowering the concentration of HDL cholesterol (Figure 2).

Small and dense LDL particles are more likely to enter the arterial wall and be oxidized, and they are thought to be the most atherogenic LDL subclass.\(^7\) Individuals whose LDL is predominately small and dense are at particularly high risk for a CV event. In the Stanford Five-City Project,\(^8\) a prospective, population-based study, LDL size was significantly smaller in those individuals who developed CHD compared with aged-matched controls. In the Quebec Cardiovascular Study,\(^9\) a prospective observational study of 2034 men without CHD, LDL size was found to be an important predictor of future CV events. Among individuals with normal levels of LDL cholesterol, the risk of a CV event was 4-fold higher in individuals with small and dense LDL particles compared with those who had large LDL particles. The relative risk of an ischemic event increased almost 7-fold in individuals who had elevated levels of LDL cholesterol in particles that were predominately small and dense.

Fibrates, niacin, and statins have all been shown to have beneficial effects on LDL size and composition. We have shown that atorvastatin at each dose significantly lowered the concentration of small and dense LDL particles and favorably increased LDL particle size.\(^10\) Higher doses of atorvastatin had more favorable effects on LDL size and concentration than did lower doses. In a post hoc analysis of the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) study\(^11\) investigating the effects of high-dose atorvastatin in patients with acute coronary syndromes, the investigators proposed that the beneficial effects of high-dose statin therapy on ischemic events were due to changes in the size and composition of LDL, rather than in changes in the concentration of LDL cholesterol. In a post hoc analysis from the Familial Atherosclerosis Treatment study,\(^12\) the change in LDL size was the most important predictor of atherosclerotic regression, more important than the change in the level of LDL cholesterol itself.

Each atherogenic lipoprotein, LDL, VLDL, IDL, and Lp(a) contains 1 protein called apolipoprotein
Lipoprotein(a)
Lp(a) is a lipoprotein that is similar to LDL. Like LDL, it contains an apo B molecule and a cholesterol-rich lipid core. However, unlike LDL, Lp(a) has a unique plasminogen-like glycoprotein, apo(a), which is linked to the LDL core by a disulfide bond. On a standard cholesterol test, the estimated LDL contains both Lp(a) and IDL particles. These are not reported separately but rather as part of the total LDL cholesterol concentration. This renders risk assessment difficult, because Lp(a) responds to therapy differently than does LDL and confers a different CHD risk. There is a strong genetic influence on the levels of Lp(a), which are higher in blacks than in Asian or white patients. The atherogenicity of Lp(a) is thought to be related to its cholesterol-rich lipid core, as well as to the prothrombotic effects related to its apo(a) moiety.14

In a large prospective study15 of 9936 healthy men and women with no prior history of CHD, an elevated Lp(a) level was associated with an increased risk of developing CHD. The higher the Lp(a) level, the greater the risk of CHD. In a meta-analysis by Danesh and colleagues,16 individuals whose Lp(a) levels ranked in the top third were at 70% increased risk of CHD compared with those with levels in the bottom third. The risk of CHD with elevated levels of Lp(a) is influenced by levels of LDL and HDL cholesterol. In the Prospective Cardiovascular Munster (PROCAM) study,17 CHD risk was only slightly increased when the Lp(a) level was elevated but LDL cholesterol and HDL cholesterol levels were normal. However, if both LDL cholesterol and Lp(a) levels were elevated, the risk of a CV event increased almost 3-fold. If the Lp(a) level was elevated and that of HDL cholesterol was low, the risk of a CV event was profoundly increased, over 8 times higher than in individuals with normal lipid levels. The finding of an elevated Lp(a) level even further increases CHD risk in an individual with elevated LDL cholesterol or low HDL cholesterol levels. The finding of an elevated Lp(a) level in these patients should lead to more aggressive treatment of their underlying lipid abnormalities.

Statins have little effect in lowering levels of Lp(a). The only lipid-lowering drug to effectively lower Lp(a) is niacin, which can on average lower levels of Lp(a) by up to 30%.18 The Familial Atherosclerosis Treatment Study19 was a 6-year study comparing 2 intensive lipid-lowering treatment strategies in high-risk men with established CHD. Study patients were randomized to conventional treatment with nonpharmacologic therapy or to 1 of 2 treatment arms: a combination of lova-statin and colestipol or niacin and colestipol. The Lovastatin/cholestipol therapy led to more regression of coronary disease and fewer CV events than did conventional treatment. Because neither lovastatin nor colestipol lowers Lp(a), little change in Lp(a) levels was seen, although the LDL cholesterol level was lowered significantly. In the niacin/cholestipol arm, similar outcome results were seen—more regression and fewer CV events than in the conventional treatment group. The LDL cholesterol level was lowered less, but that of Lp(a) was lowered more than in patients randomized to lovastatin and colestipol. The results from this study suggest that coronary atherosclerotic progression and CV event rate can be reduced in patients with elevated levels of Lp(a) by intensive LDL cholesterol lowering with statin therapy or by targeting Lp(a) directly with niacin.

Triglyceride-Rich Remnant Lipoproteins
Recent studies have shown that elevated levels of serum triglycerides are an independent risk factor for cardiovascular disease (CVD). The triglyceride-rich remnant lipoproteins are remnants of very low-density lipoprotein (VLDL) particles by the liver. The TGs within the largest VLDL subclass are exchanged for cholesteryl esters (CEs) on large LDL particles, as well as high-density lipoprotein (HDL) particles by CE transfer protein (CETP). This enriches both LDL and HDL particles with TGs. TGs within LDL particles are hydrolyzed by hepatic lipase, producing a CE-depleted, small, dense LDL particle. As TGs within HDL particles are hydrolyzed by hepatic lipase, smaller HDL particles are produced that contain less CE. These smaller HDL particles are more avidly taken up and removed by the kidney, leading to lower concentrations of HDL cholesterol in the blood.
Figure 3. When triglyceride (TG) levels are low, very low-density lipoprotein (VLDL) particles are smaller and less rich in TGs. These smaller particles undergo delipidation by lipoprotein lipase (LPL) to intermediate-density lipoprotein (IDL) and subsequently to low-density lipoprotein (LDL), which is predominately rich in cholesteryl ester and large in size. When TG levels are elevated, very large TG-rich VLDL particles are formed. Delipidation by LPL is incomplete, leading to the accumulation of smaller VLDL and IDL remnant lipoproteins. Lipolysis by LPL and hepatic lipase, along with further TG enrichment by cholesteryl ester transfer protein (CETP) of the LDL particle, leads to the formation of smaller LDL particles. Adapted with permission from Berneis and Krauss.20

for CHD.1 The increased CV risk associated with hypertriglyceridemia is thought to be due in part to increased levels of the triglyceride-rich lipoproteins, VLDL and IDL cholesterol. High levels of triglycerides lead to the production of large VLDL particles. Degradation of these large triglyceride-rich VLDL particles by lipoprotein lipase results in small VLDL and IDL remnants which are particularly atherogenic; these are defined by the NCEP ATP III guidelines as remnant lipoproteins and are considered an emerging lipid-related risk factor (Figure 3). The presence of elevated levels of small and dense LDL cholesterol particles and low levels of HDL cholesterol, often found in persons with hypertriglyceridemia, adds further to CV risk. These lipid abnormalities commonly coexist in patients with type 2 diabetes or the metabolic syndrome.20

Reducing levels of triglyceride-rich remnant lipoproteins is clinically important because of their atherogenic potential. In clinical studies,20 both small VLDL (VLDL₃) and IDL cholesterol have been shown to be associated with atherosclerotic progression and increased CV risk, independent of total levels of fasting triglycerides. Any lipid-lowering therapy that lowers triglyceride levels will also lower levels of triglyceride-rich remnant lipoproteins. In one study,10 high-dose atorvastatin (80 mg/dL) reduced the levels of VLDL₃ and IDL by 55%, achieving normal levels of these remnant lipoproteins in >80% of patients with triglyceride levels >200 mg/dL. The primary lipid effect of fibrates is the lowering of triglyceride levels. Triglyceride-rich remnant lipoproteins are lowered by up to 35% with fenofibrate.21 In clinical studies, the combination of statin and niacin has lowered levels of VLDL by 39% and IDL by 57%.18

HDL Subclasses

Individuals with low HDL cholesterol levels, <40 mg/dL for men and <50 mg/dL for women, are at increased risk for CHD.1 As with LDL cholesterol, HDL cholesterol particles vary in size and composition. HDL is categorized into 2 major subclasses: HDL₂, which is the larger particle and more enriched in cholesterol, and HDL₃, which is smaller and has lower cholesterol content. The levels of HDL₂ are thought to be a measure of reverse cholesterol transport, with higher levels reflecting more efficient reverse cholesterol transport.

Although HDL subclasses have been less studied than LDL subclasses, several studies have demonstrated that the measurement of HDL subclasses can provide useful information regarding CHD risk beyond that obtained from established risk factors. Asztalos and colleagues22 measured HDL subclass concentrations in a subset of patients from the Framingham Offspring Study. The measurement of larger HDL particles better differentiated those individuals who developed CHD from controls than did levels of either HDL or LDL cholesterol. In the Veterans Affairs HDL Intervention Trial (VA-HIT),23 a study evaluating the fibrate gemfibrozil in men
with CHD and low levels of HDL cholesterol, levels of larger HDL particles were superior to levels of HDL cholesterol in predicting CV risk. The evidence suggests that larger HDL₃ is the more protective HDL subclass and that higher levels of HDL₂ are associated with less atherosclerotic progression and a lower risk of CV events. Although statins and fibrates can raise the HDL cholesterol level, nicin is the most effective drug in increasing levels of HDL cholesterol. In the HDL Atherosclerosis Treatment Study, men with CHD treated with a combination of simvastatin and nicin had significantly greater increases in levels of large HDL particles (HDL₂ levels increased by 65%) and significantly less coronary artery disease progression and fewer CV events than patients randomized to placebo. Statins have modest effects in raising the level of HDL cholesterol and preferentially raise levels of HDL₂. In a study of patients with hypertriglyceridemia, low-dose atorvastatin raised levels of HDL₂ by 4%, while the higher 80-mg dose raised HDL₂ by 10%. Fibrates raise levels of HDL cholesterol, but unlike statins and nicin, do so primarily by raising levels of the smaller HDL₃ subclass.

INTEGRATING ADVANCED CHOLESTEROL TESTING INTO CLINICAL PRACTICE

Screening for established CV risk factors and calculating the Framingham risk score remain the foundation for CHD risk assessment, as well as determining the need for and intensity of lipid-lowering therapy. However, relying solely on the Framingham risk score to predict CV risk has limitations, especially in women and individuals with a family history of premature CHD. In the Healthy Women Study, coronary artery calcium and lipoprotein subclasses were measured in a cohort of 286 postmenopausal women. Small LDL and large VLDL particles were positively associated with the presence of coronary calcium, even after adjusting for established CV risk factors and standard measurements of LDL cholesterol, HDL cholesterol, and triglyceride levels. The authors concluded that the measurement of lipid subclasses may improve the prediction of CHD over a standard cholesterol test in otherwise healthy postmenopausal women. We recently evaluated 69 healthy younger men and women with a low Framingham risk score and a family history of premature CHD. More than one-third had evidence of subclinical atherosclerosis based on a positive coronary calcium score, and there was a high prevalence of emerging lipid-related risk factors. A low level of HDL₂ was present in 74% of patients and was the most common emerging lipid-related risk factor found. Other studies have found no benefit of advanced cholesterol testing compared with the standard cholesterol test in predicting CV risk. In a group of asymptomatic young adults from the Bogalusa Heart Study, advanced cholesterol testing did not predict carotid intima-media thickness better than the standard cholesterol test did.

CONCLUSIONS

The NCEP ATP III guidelines recommend that when evaluating an individual’s CHD risk, this assessment should be based on the number of major risk factors present. However, the guidelines recognize the clinical value of the emerging lipid-related risk factors Lp(a), remnant lipoproteins, and small LDL cholesterol particles. The guidelines state that emerging lipid risk factors “can be taken into consideration according to clinical judgment as optional modifiers of therapy.” Although advanced lipid testing cannot be recommended for routine screening, it may be of value in certain groups of at-risk patients. These include individuals with a family history of premature CHD, postmenopausal women, and individuals at intermediate risk for CHD, especially if they are near the boundary of being at high risk. For these at-risk patients, the presence of emerging lipid risk factors may provide the clinician support to shift that individual to a higher risk category and a lower LDL cholesterol level goal. For example, it is reasonable for a clinician to aim for an LDL cholesterol goal <100 mg/dL, rather than <130 mg/dL, in an individual with a family history of premature CHD and an intermediate Framingham risk score whose Lp(a) is elevated or whose LDL cholesterol is predominately small and dense (Figure 4).

The guidelines from NCEP ATP III focus on dietary and lifestyle changes as an integral part of reducing CHD risk. Weight loss and exercise have been shown not only to improve LDL₃, HDL, and triglyceride levels but also to favorably shift LDL particle size, raise levels of large HDL particles, and reduce levels of triglyceride-rich remnant lipoproteins. If lifestyle changes are unsuccessful in
achieving the lower LDL cholesterol goal in a patient with emerging lipid risk factors, then consideration should be given to initiate or intensify pharmacologic lipid-lowering therapy. Although no studies have shown all-cause mortality and morbidity reduction in treating any emerging risk factors, there is no doubt that their presence enhances CHD risk at any level. This reinforces the need to focus on LDL cholesterol as the target for lipid-lowering therapy in those patients with known abnormal emerging risk factors. Non–HDL cholesterol remains a secondary target of therapy. Ongoing studies will better define the role of lipid subclasses and advanced cholesterol testing in clinical practice. For now, the tests play a valuable role as an adjunct to traditional risk factors to assess CV risk.

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REFERENCES