Medical Policy

BlueCross BlueShield of Kansas
An Independent Licensee of the Blue Cross and Blue Shield Association

Title: Novel Lipid Risk Factors in Risk Assessment and Management of Cardiovascular Disease

Professional
Original Effective Date: March 13, 2009
Revision Date(s): February 10, 2011; September 20, 2011; September 18, 2012
Current Effective Date: February 10, 2011

Institutional
Original Effective Date: March 13, 2009
Revision Date(s): February 10, 2011; September 20, 2011; September 18, 2012
Current Effective Date: February 10, 2011

DESCRIPTION

Numerous lipid and nonlipid biomarkers have been proposed as potential risk markers for cardiovascular disease. This policy will focus on those lipid markers that have the most evidence in support of their use in clinical care. The lipid markers assessed here are apolipoprotein B, apolipoprotein A-1, high-density lipoprotein (HDL) subclass, low-density lipoprotein (LDL) subclass, apolipoprotein E, and lipoprotein A.

Background

Low-density lipoproteins (LDL) have been identified as the major atherogenic lipoproteins and have long been identified by the National Cholesterol Education Project (NCEP) as the primary target of cholesterol- lowering therapy. LDL particles consist of a surface coat composed of phospholipids, free cholesterol, and apolipoproteins surrounding an inner lipid core composed of cholesterol ester and triglycerides. Traditional lipid risk factors such as LDL-cholesterol (LDL-C), while predictive on a population basis, are weaker markers of risk on an individual basis. Only a minority of subjects with elevated LDL and cholesterol levels will develop clinical disease, and up to 50% of cases of coronary artery disease (CAD) occur in subjects with 'normal' levels of total and LDL-C. Thus, there is considerable potential to improve the accuracy of current cardiovascular risk prediction models.

Apolipoprotein B. Apolipoprotein B (apo B) is the major protein moiety of all lipoproteins except for high-density lipoprotein (HDL). The most abundant form of apo B, large B or B-100, constitutes the apo B found in LDL and very-low-density lipoproteins (VLDL). Since both LDL and VLDL each contain one molecule of apolipoprotein B, measurement of apo B reflects the total number of these atherogenic particles, 90% of which are LDL. Since LDL particles can vary both in size and in cholesterol content, for a given concentration of LDL-C, there can be a wide variety of both size and numbers of LDL particles. Thus, it has been postulated that apo B is a better measure of the atherogenic potential of serum LDL than is LDL concentration.

Two basic techniques are used for measuring LDL particle concentration. Particle size can be determined by gradient gel electrophoresis, or direct measurement of the number of LDL particles can be performed using nuclear magnetic spectroscopy. Nuclear magnetic resonance (NMR) spectroscopy is based on the fact that lipoprotein subclasses of different size broadcast distinguishable NMR signals. Thus NMR can quantify the number of LDL particles of a specific size (i.e., small dense LDL) and can provide a measurement of the total number of particles.

Apolipoprotein A-I. HDL contains two associated apolipoproteins, i.e., A-I and A-II. HDL particles can also be classified by whether they contain apolipoprotein A-I (apo A-I) only or whether they contain both apo A-I and apolipoprotein A-II (A-II). All lipoproteins contain apo A-I, and some also contain apo A-II. Since all HDL particles contain apo A-I, this lipid marker can be used as an approximation for HDL number, similar to the way apo B has been proposed as an approximation of the LDL number.

Direct measurement of apo A-I has been proposed as more accurate than the traditional use of HDL level in evaluation of the cardioprotective, or "good," cholesterol. In addition, the ratio of apolipoprotein B (apo B)/apo A-I has been proposed as a superior measure of the ratio of proatherogenic (i.e., "bad") cholesterol to anti-atherogenic (i.e., "good") cholesterol.

Apolipoprotein E. Apolipoprotein E (apo E) is the primary apolipoprotein found in VLDLs and chylomicrons. Apo E is the primary binding protein for LDL receptors in the liver and is thought to play an important role in lipid metabolism. The apo E gene is polymorphic, consisting of 3 alleles (e2, e3, and e4) that code for 3 protein isoforms, known as E2, E3, and E4, which differ from one another by one amino acid. These molecules mediate lipid metabolism through their different interactions with the LDL receptors. The genotype of apo E alleles can be assessed by gene amplification techniques, or the apo E phenotype can be assessed by measuring plasma levels of apo E.

It has been proposed that various apo E genotypes are more atherogenic than others and that apo E measurement may provide information on risk of coronary artery disease (CAD) above traditional risk factor measurement. It has also been proposed that the apo E genotype may be useful in the selection of specific components of lipid-lowering therapy, such as drug selection. In the major lipid-lowering intervention trials, including trials of statin therapy, there is considerable variability in response to therapy that cannot be explained by factors such as compliance. Apo E genotype may be one factor that determines an individual's degree of response to interventions such as statin therapy.

LDL subclass. Two main subclass patterns of LDL, called A and B, have been described. In subclass pattern A, the particles have a diameter larger than 25 nm and are less dense, while in subclass pattern B, the particles have a diameter less than 25 nm and a higher density. Subclass pattern B is a commonly inherited disorder associated with a more atherogenic lipoprotein profile, also termed "atherogenic dyslipidemia." In addition to small, dense LDL, this pattern includes elevated levels of triglycerides, elevated levels of apolipoprotein B, and low levels of HDL. This lipid profile is commonly seen in type II diabetes and is one component of the "metabolic syndrome," defined by the Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III; ATP III) to also include high normal blood pressure, insulin resistance, increased levels of inflammatory markers such as C-reactive protein (CRP), and a prothrombotic state. Presence of the metabolic syndrome is considered by ATP III to be a substantial risk-enhancing factor for CAD.

LDL size has also been proposed as a potentially useful measure of treatment response. Lipid-lowering treatment decreases total LDL and may also induce a shift in the type of LDL, from smaller, dense particles to larger particles. It has been proposed that this shift in lipid profile may be beneficial in reducing risk for CAD independent of the total LDL level. Also, some drugs may cause a greater shift in lipid profile than others. Niacin and/or fibrates may cause a greater shift from small to large LDL size than statins. Therefore, measurement of LDL size may potentially play a role in drug selection or may be useful in deciding to use a combination of 2 or more drugs rather than a statin alone.

In addition to the size of LDL particles, interest has been shown in assessing the concentration of LDL particles as a distinct cardiac risk factor. For example, the commonly performed test, LDL-C is not a direct measure of LDL but, chosen for its convenience, measures the amount of cholesterol incorporated into LDL particles. Since LDL particles carry much of the cholesterol in the bloodstream, the concentration of cholesterol in LDL correlates reasonably well with the number of LDL particles when examined in large populations. However, for an individual patient, the LDL-C level may not reflect the number of particles due to varying levels of cholesterol in different sized particles. It is proposed that the discrepancy between the number of LDL particles and the serum level of LDL-C represents a significant source of unrecognized atherogenic risk. The size and number of particles are interrelated. For example, all LDL particles can invade the arterial wall and initiate atherosclerosis. However, small, dense particles are thought to be more atherogenic compared to larger particles. Therefore, for patients with elevated numbers of LDL particles, cardiac risk may be further enhanced when the particles are smaller versus larger.

Two techniques are most commonly used for measuring LDL particle concentration, the surrogate measurement of apo B or direct measurement of the number of particles using NMR. NMR is used based on the fact that lipoprotein subclasses of different size broadcast distinguishable NMR signals. Thus NMR can directly measure the number of LDL particles of a specific size (i.e., small dense LDL) and can provide a measurement of the total number of particles. Thus, NMR is proposed as an additional technique to assess cardiac risk.

HDL subclass. HDL particles exhibit considerable heterogeneity, and it has been proposed that various subclasses of HDL may have a greater role in protection from atherosclerosis. Particles of HDL can be characterized based on size/density and/or on the apolipoprotein composition. Using size/density, HDL can be classified into HDL2, the larger, less dense particles that may have the greatest degree of cardioprotection, and HDL3, which are smaller, more dense particles. HDL contains 2 associated apolipoproteins, i.e., A-I and A-II. HDL particles can also be classified by whether they contain apolipoprotein A-I (apo A-I) only or whether they contain both apo A-I and apolipoprotein A-II (apo A-II). There has been substantial interest in determining whether subclasses of HDL can be used to provide additional information on cardiovascular risk compared to HDL alone.

An alternative to measuring the concentration of subclasses of HDL, such as HDL2 and HDL3, is direct measurement of HDL particle size and/or number. Particle size can be measured by NMR spectroscopy or by gradient-gel electrophoresis. HDL particle numbers can be measured by NMR spectroscopy. Several commercial labs offer these measurements of HDL particle size and number. Measurement of apo A-I has used measurement of HDL particle number as a surrogate, based on the premise that each HDL particle contains one apo A-I molecule.

Lipoprotein A. Lipoprotein (a) (lp[a]) is a lipid-rich particle similar to LDL. Apolipoprotein B is the major apolipoprotein associated with LDL; in lp(a), however, there is an additional apolipoprotein A covalently linked to the apolipoprotein B. The apolipoprotein (a) molecule is structurally similar to plasminogen, suggesting that lp(a) may contribute to the thrombotic and atherogenic basis of cardiovascular disease. Levels of lp(a) are relatively stable in individuals over time, but vary up to 1,000-fold between individuals, presumably on a genetic basis. The similarity between lp(a)and fibrinogen has stimulated intense interest in lp(a) as a link between atherosclerosis and thrombosis. In addition, approximately 20% of patients with CAD have elevated levels of lp(a). Therefore, it has been proposed that levels of lp(a) may be an independent risk factor for CAD.

POLICY

Measurement of novel lipid risk factors (i.e., apolipoprotein B, apolipoprotein A-I, apolipoprotein E, LDL subclass, HDL subclass, lipoprotein[a]) is considered **experimental/ investigational** as an adjunct to LDL cholesterol in the risk assessment and management of cardiovascular disease.

RATIONALE

Introduction

A large body of literature has accumulated on the utility of novel lipid risk factors in the prediction of future cardiac events. The evidence reviewed for this policy statement consists of large, prospective cohort studies that have evaluated the association of these lipid markers with cardiovascular outcomes. A smaller amount of literature is available on the utility of these markers as a marker of treatment response. Data on treatment response is taken from randomized, controlled trials (RCTs) that use one or more novel lipid markers as a target of lipid-lowering therapy.

The ATP III guidelines document (1) notes that to determine their clinical significance, the emerging risk factors should be evaluated against the following criteria in order to determine their clinical significance:

- Significant predictive power that is independent of other major risk factors
- A relatively high prevalence in the population (justifying routine measurement in risk assessment)
- Laboratory or clinical measurement must be widely available, well standardized, inexpensive, have accepted population reference values, and be relatively stable biologically
- Preferable, but not necessarily, modification of the risk factor in clinical trials will have shown reduction in risk.

A 2002 TEC Assessment (2) summarized the steps necessary to determine utility of a novel cardiac risk factor. Three steps were required:

1. Standardization of the measurement of the risk factor.
2. Determination of its contribution to risk assessment. As a risk factor, it is important to determine whether the novel risk factor [...] independently contributes to risk assessment compared to established risk factors.
3. Determination of how the novel risk assessment will be used in the management of the patient, compared to standard methods of assessing risk, and whether any subsequent changes in patient management result in an improvement in patient outcome.

Each of the individual novel lipid risk factors will be judged individually against these criteria to determine whether health outcomes are improved through measurement of the novel lipid risk factor.

Apolipoprotein B

Apo B as a Predictor of Cardiovascular Risk. The Emerging Risk Factors Collaboration published a patient-level meta-analysis of 37 prospective cohort studies enrolling 154,544 individuals. (3) Risk prediction was examined for a variety of traditional and non-traditional lipid markers. For apolipoprotein B (apo B), evidence from 26 studies on 139,581 individuals reported that apo B was an independent risk factor for cardiovascular events, with an adjusted hazard ratio of 1.24 (95% confidence interval [CI]: 1.19-1.29). On reclassification analysis, when apo B and apo AI were substituted for traditional lipids, there was not improvement in risk prediction. In fact, there was a slight worsening in the predictive ability, evidenced by a decrease in the C-statistic of -0.0028 ($p<0.001$), and a decrease in the net reclassification improvement of -1.08% ($p<0.01$).

The Quebec Cardiovascular Study (4) evaluated the ability of levels of apo B and other lipid parameters to predict subsequent coronary artery disease (CAD) events in a prospective cohort study of 2,155 men followed up for 5 years. Elevated levels of apo B were found to be an independent risk factor for ischemic heart disease after adjustment for other lipid parameters (risk ratio [RR]: 1.40; 95% CI: 1.2–1.7). In patients with an apo B level of greater than 120 mg/dL, there was a 6.2-fold increase in the risk of cardiovascular events.

The Apolipoprotein Mortality Risk Study (AMORIS) (5) was another prospective cohort study that followed up 175,000 Swedish men and women presenting for routine outpatient care over a mean of 5.5 years. This study found that apo B was an independent predictor of CAD events and was superior to low-density lipoprotein-cholesterol (LDL-C) levels in predicting risk, both for the entire cohort and in all subgroups examined. Risk ratios for the highest quartile of apo B levels were 1.76 in men ($p<0.0001$) and 1.69 in women ($p<0.001$).

A cohort study of 15,632 participants from the Women's Health Initiative (6) provided similar information in women. In this analysis, the hazard ratio for developing coronary heart disease in the highest versus the lowest quintiles was greater for apo B (2.50; 95% CI: 1.68–3.72) compared to LDL-C (1.62; 95% CI: 1.17–2.25), after adjustment for traditional cardiovascular risk factors.

The Copenhagen City Heart Study (7) was a prospective cohort study of 9,231 asymptomatic persons from the Danish general population followed up for 8 years. Individuals with total apo B levels in the top one-third (top tertile) had a significantly increased relative risk of cardiovascular events compared to patients in the lowest one-third, after controlling for LDL-C and other traditional cardiovascular risk factors (RR: 1.4, 95% CI: 1.1–1.8 for men; RR: 1.5, 95% CI: 1.1–2.1 for women). This study also compared the discriminatory ability of apo B with that of traditional lipid measures, by using the area under the curve (AUC) for classifying cardiovascular events. Total apo B levels had a slightly higher AUC compared to LDL-C (0.58 vs. 0.57, respectively); however this difference in AUC was not statistically significant.

At least one large prospective cohort study, the Atherosclerosis Risk in Communities (ARIC) study, (8) concluded that apo B did not add additional predictive information above standard lipid measures. The ARIC study followed up 12,000 middle-aged individuals free of CAD at baseline for 10 years. While apo B was a strong univariate predictor of risk, it did not add independent predictive value above traditional lipid measures in multivariate models.

The ratio of apo B/apo A-I has also been proposed as a superior measure of the ratio of pro-atherogenic (i.e., “bad”) cholesterol to anti-atherogenic (i.e., “good”) cholesterol. This ratio may be a more accurate measure of this concept, compared to the more common total cholesterol/high-density lipoprotein (TC/HDL) ratio. A number of epidemiologic studies have reported that the apo B/apo A-I ratio is superior to other ratios, such as TC/HDL-C, or non-high-density lipoprotein-cholesterol (HDL-C)/HDL-C. (9, 10)

Kappelle et al. (11) used data from the prospective PREVEND cohort to evaluate the predictive value of the apo B/apo A-1 ratio independent of other traditional risk factors, including albuminuria and C-reactive protein (CRP). Among 6,948 individuals without previous heart disease and who were not on lipid-lowering drugs, the adjusted hazard ratio for a high apo B/apo A-1 ratio was 1.37 (95% CI: 1.26-1.48). This hazard ratio was not significantly different from the total cholesterol/HDL-C ratio of 1.24 (95% CI: 1.18-1.29), and was not significantly changed after further adjustment for triglycerides.

Some studies have tested the use of apo B in a multivariate risk prediction model in which both traditional risk factors and apolipoprotein measures were included as potential predictors. Ridker and co-workers (12) published the Reynolds Risk Score, based on data from 24,558 initially healthy women enrolled in the Women’s Health Study and followed up for a median of 10.2 years. A total of 35 potential predictors of cardiovascular disease were considered as potential predictors, and 2 final prediction models were derived. The first model was the best fitting model statistically, and included both apo B and the apo B/apo A-I ratio as 2 of 9 final predictors. The second model, called the “clinically simplified model,” substituted LDL-C for apo B and total/high-density lipoprotein (HDL) cholesterol for apo B/apo A-I. The authors developed this simplified model “for the purpose of clinical application and efficiency” and justified replacing the apo-B and apo B/apo A-I measures as a result of their high correlation with traditional lipid measures (r=0.87 and 0.80, respectively).

Ingelsson and co-workers (13) used data from 3,322 individuals in the Framingham Offspring Study to compare prediction models with traditional lipid measures to models that include apolipoprotein and other nontraditional lipid measures. This study reported that the apo B/apo A-I ratio had similar predictive ability as traditional lipid ratios with respect to model discrimination, calibration, and reclassification. The authors also reported that the apo B/apo A-I ratio did not provide any incremental predictive value over traditional measures.

Apo B as a Treatment Target. A number of RCTs of statin therapy have examined the change in apo B on treatment in relation to clinical CAD outcomes and compared whether apo B is a better predictor of outcomes when compared to LDL-C.

The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) evaluated lipid parameters among 6,605 men and women with average LDL- and low HDL-cholesterol levels who were randomly assigned to receive either lovastatin or placebo. (14) Baseline LDL- and HDL-cholesterol, as well as levels of apo B were predictive of future coronary events. However, in the treatment group, post-treatment levels of LDL-C and HDL-C were not predictive of subsequent risk, while post-treatment apo-B levels were predictive.

In the Long-Term Intervention with Pravastatin in Ischemic Disease (LIPID) trial, (15) the relationship of on-treatment apo B levels to clinical outcomes was examined in 9,140 patients randomized to pravastatin or placebo and followed up for a mean of 6.1 years. The adjusted hazard ratio for apo B levels (2.10; 95% CI: 1.21–3.64, p=0.008) was higher than that for LDL-C (1.20; 95% CI: 1.00–1.45, p=0.05). Also, the proportion of the treatment effect explained by on-treatment apo B levels (67%) was higher than that for LDL-C levels (52%).

Kastelein et al. (16) combined data from 2 RCTs, the Treating to New Targets (TNT) and Incremental Decrease in End Points through Aggressive Lipid Lowering (IDEAL) trials, to compare the relationship between response to lipids, apo B levels, and other lipid measures. This analysis included 18,889 patients with established coronary disease randomly assigned to low- or high-dose statin treatment. In pairwise comparisons, the on-treatment apo B level was a significant predictor of cardiovascular events (HR: 1.24; 95% CI: 1.13–1.36, p<0.001), while LDL level was not. Similarly, the ratio of apo B/apo A-I was a significant predictor of events (HR: 1.24; 95% CI: 1.17–1.32), while the total/HDL-C was not. In another publication that reported on the TNT study (17), the on-treatment apo B level was also a significant predictor of future events (adjusted hazard ratio 1.19, 95% CI 1.11-1.28). In this study, the known baseline variables performed well in discriminating future cases from non-cases, and the addition of apo B was not associated with additional risk.

Mora et al. (18) measured on-treatment lipid levels to assess the prediction of residual risk while on statin therapy. Using data from the JUPITER trial, on-treatment levels of LDL-C, non-HDL cholesterol, hs (high-sensitivity)-CRP, apo B, and apo A-I were used to predict subsequent cardiovascular events. The hazard ratios for cardiovascular events were similar among all the lipid measures, ranging from 1.22 to 1.31, with no significant differences between measures. The residual risk declined overall with a decreasing level of LDL-C, with the lowest risk seen in individuals achieving an LDL-C of less than 70mg/dL.

Boekholdt et al. (19) published an individual patient-level meta-analysis of on-treatment levels of traditional and non-traditional lipids as a measure of residual risk. A total of 8 studies enrolling 62,154 participants were included. The adjusted hazard ratio for each 1 standard deviation (SD) increase in apo B was 1.14 (95% CI: 1.11-1.18), which was not significantly different from LDL-C (hazard ratio [HR}: 1.13, 95% CI: 1.10-1.17, p=0.21). The hazard ratio for HDL-C was 1.16 (95% CI: 1.12-1.19), which was significantly greater than LDL-C or apo B (p=0.002).

<u>Current Treatment Guidelines</u>. The ATP III guidelines (1) identify apo B as an "emerging risk factor." In their discussion of apo B, the guidelines state that the apo B level typically is disproportionately higher in persons with hypertriglyceridemia, and that "ATP III takes this difference into account and sets a secondary target, non HDL cholesterol, in persons with hypertriglyceridemia. Non-HDL cholesterol is significantly correlated with apolipoprotein B and can serve as a 'surrogate' for it. The non-HDL cholesterol measure is readily available in clinical practice, whereas standardized apolipoprotein B measures are not widely available."

In 2004, the American College of Physicians published clinical practice guidelines regarding lipid control in the management of type 2 diabetes. (20) These guidelines do not address the role of measurement of either apo B or direct measurements of lipid particle concentration. In July 2004, Grundy and colleagues published an article outlining the implications of clinical trials of statin therapy that were published after ATP III. (21) The authors recommended a further lowering of the target LDL-C for some populations of patients. For example, the LDL-C target of 100 mg/dL in high-risk patients was lowered to 70 mg/dL, and the target in moderately high-risk patients was lowered from 130 to 100. These more aggressive targets of therapy create additional questions of how measurements of apo B can be used to improve patient management.

A publication from a consensus conference of the American Diabetes Association and the American College of Cardiology Foundation (22) included specific recommendations for incorporating apo B testing into clinical care for high-risk patients. This expert panel stated that "ApoB and LDL particle number also appear to be more discriminating measures of the adequacy of LDL lowering therapy than are LDL cholesterol or non-HDL cholesterol." They therefore recommend that for patients with metabolic syndrome who are being treated with statins, both LDL-C and apo B should be used as treatment targets, with an apo B target of less than 90 mg/dL. Treatment should be intensified for patients with apo B above this level, even if target LDL has been achieved.

A Canadian task force has also endorsed use of apo B as a treatment target and proposed a target apo B level of 90 mg/dL. (23) Other experts have recommended using a lower target of 80 mg/dL for apo B. (24) However, none of the major guideline entities in the United States, such as ATP III, have incorporated apo B targets as part of their formal recommendations.

<u>Conclusions</u>. The evidence suggests that apo B provides independent information on risk assessment for cardiovascular disease and that apo B is superior to LDL-C in predicting cardiovascular risk. Numerous large prospective cohort studies and nested case-control studies have compared these measures, and most have concluded that apo B is a better predictor of cardiac risk when compared to LDL-C. There is greater uncertainty around the degree of improvement in risk prediction and whether the magnitude of improvement is clinically significant. While there have been attempts to incorporate apo B into multivariate risk prediction models, at the present time, apo B is not included in the models that are most commonly used in routine clinical care, such as the Framingham risk model and the Prospective Cardiovascular Munster Study (PROCAM) Score.

Furthermore, as a marker of response to cholesterol-lowering treatment, apo B may be more accurate than LDL-C and may provide a better measure of the adequacy of anti-lipid therapy than does LDL-C. Post-hoc analyses of RCTs of statin treatment have reported that on-treatment levels of apo B are more highly correlated with clinical outcomes than standard lipid measures. Whether the degree of improvement in assessing treatment response is clinically significant has yet to be determined.

Some experts currently believe that the evidence is sufficient to warrant the routine clinical use of apo B levels as a replacement for LDL-C levels and the use of the apo B/apo A-I ratio as a replacement for the TC/HDL-C ratio. These experts argue that the use of apo B in place of LDL-C will allow better targeting of anti-lipid therapy and avoid undertreatment in a substantial number of patients with low or normal LDL levels and small, dense subtype (high apo B). As of the current time, none of the major guidelines, such as ATP III, have yet to formally incorporate the measurement of apo B into their recommendations.

However, it is not yet possible to conclude that the use of apo B levels will improve outcomes when used in routine clinical care. Improved ability to predict risk and/or treatment response does not by itself result in better health outcomes. To improve outcomes, clinicians must have the tools to translate this information into clinical practice. No studies have demonstrated improved health outcomes by using apo B in place of LDL-C for either risk assessment and/or treatment response. The most widely used risk assessment models, such as the Framingham prediction model, and the most widely used treatment guidelines, the ATP III guidelines, do not provide the tools necessary for clinicians to incorporate apo B measurements into routine assessment and management of hyperlipidemic patients. This lack creates difficulties in interpreting and applying the results of apo B and/or apo B/apo A-I measurements to routine clinical care.

Therefore, based on review of the currently available evidence, this testing is considered investigational.

Apolipoprotein A-I (apo A-I)

Apo A-I as a Predictor of Cardiovascular Disease. The Emerging Risk Factors Collaboration published a patient-level meta-analysis of 37 prospective cohort studies enrolling 154,544 individuals. (3) Risk prediction was examined for a variety of traditional and non-traditional lipid markers. For apo A-I, evidence from 26 studies on 139,581 individuals reported that apo A-I was an independent risk factor for reduced cardiovascular risk, with an adjusted hazard ratio for cardiovascular events of 0.87 (95% CI: 0.84-0.90). On reclassification analysis, when apo B and apo A-I were substituted for traditional lipids, there was not improvement in risk prediction. In fact, there was a slight worsening in the predictive ability, evidenced by a decrease in the C-statistic of -0.0028 ($p<0.001$) and a decrease in the net reclassification improvement of -1.08% ($p<0.01$).

The Apolipoprotein-Related Mortality Risk Study (AMORIS) followed up 175,000 Swedish men and women for 5.5 years (5) and reported that decreased apo A-I was an independent predictor of coronary artery disease (CAD) events. The AFCAPS/TexCAPS investigated lipid parameters among 6,605 men and women with average low-density lipoprotein cholesterol (LDL-C) and low HDL-cholesterol who were randomized to receive either lovastatin or placebo. (14) This study also reported that levels of apo A-I, as well as the ratio of apo B/apo A-I, were strong predictors of CAD events.

The Copenhagen City Heart Study (7) was a prospective cohort study of 9,231 asymptomatic persons from the Danish general population. The apo B/apo A-I ratio was reported to be an independent predictor of cardiovascular events, with a hazard ratio similar to that for total cholesterol/HDL cholesterol. This study also compared the discriminatory ability of the apo B/apo A-I ratio with that of traditional lipid measures, with use of the AUC for classifying cardiovascular events. The apo B/apo A-I ratio had a slightly higher AUC when compared to total cholesterol/HDL cholesterol ratio (0.59 vs. 0.58, respectively), but this difference was not statistically significant.

Clarke and colleagues (25) published a prospective cohort study of 7,044 elderly men enrolled in the Whitehall Cardiovascular Cohort from London, England. Measurements of apolipoprotein levels were performed on 5,344 of these individuals, and patients were followed up for a mean of 6.8 years. The authors reported that the apo B/apo A-I ratio was also a significant independent predictor (hazard ratio [HR] 1.54; 95% CI: 1.27–1.87), with similar predictive ability compared to the total cholesterol/HDL ratio (HR 1.57; 95% CI: 1.32–1.86).

The addition of the apo B/apo A-I ratio to the Framingham risk model (26) resulted in a statistically significant improvement in predictive value for cardiovascular events (AUC 0.594 vs. AUC 0.613, respectively; p<001). However, the authors concluded that this increment in predictive value was likely to be of little clinical value. This same study also reported the predictive ability of apo A-II in a separate publication. In this analysis, individuals with apo A-II levels in the highest quartile had a decreased risk of cardiovascular events compared to those in the lowest quartile (adjusted odds ratio [OR]: 0.62; 95% CI: 0.43–0.90).

Ridker et al. (12) compared the predictive ability of apo A-I and the ratio of apo B/apo A-I to standard lipid measurements. Measurements of apo A-I and the apo B/apo A-I ratio had similar predictive ability to standard lipid measurements but were no better. The hazard ratio for future cardiovascular events was 1.75 (95% CI: 1.30–2.38) for apo A-I, compared to 2.32 (95% CI: 1.64–3.33) for HDL-C. The hazard ratio for the ratio of apo B/apo A-I was 3.01 (95% CI: 2.01–4.50), compared with a hazard ratio of 3.18 (95% CI: 2.12–-4.75) for the ratio of LDL-C/HDL-C.

Some researchers have attempted to develop multivariate risk prediction models intended for use in clinical care, in which both traditional risk factors and apolipoprotein measures were included as potential predictors. Ridker and colleagues (12) published the Reynolds Risk Score, based on data from 24,558 initially healthy women enrolled in the Women's Health Study and followed up for a median of 10.2 years. A total of 35 potential predictors of cardiovascular disease were considered potential predictors, and 2 final prediction models were derived. The first model was the best-fitting model statistically and included both apo B and the apo B/apo A-I ratio as 2 of 9 final predictors. The second model, called the "clinically simplified model," substituted LDL-C for apo B and total cholesterol/HDL cholesterol for apo B/apo A-I. The authors developed this simplified model "for the purpose of clinical application and efficiency" and justified replacing the apo B and apo B/apo A-I measures as a result of their high correlation with traditional lipid measures (r=0.87 and 0.80, respectively).

Ingelsson and colleagues (13) used data from 3,322 individuals in the Framingham Offspring Study to compare prediction models with traditional lipid measures to models that include apolipoprotein and other nontraditional lipid measures. This study reported that the apo B/apo A-I ratio had similar predictive ability compared to traditional lipid ratios with respect to model discrimination, calibration, and reclassification. The authors also reported that the apo B/apo A-I ratio did not provide any incremental predictive value over traditional measures.

A nested case-control study, (27) performed within the larger EPIC-Norfolk cohort study, evaluated the predictive ability of apo B/apo A-I in relation to traditional lipid measures. The European Prospective Investigation into Cancer and Nutrition-Norfolk (EPIC-Norfolk) study is a cohort study of 25,663 patients from Norfolk, U.K. The case control substudy enrolled 869 patients who had developed CAD during a mean follow-up of 6 years and 1,511 control patients without CAD. The authors reported that the apo B/apo A-I ratio was an independent predictor of cardiovascular events after controlling for traditional lipid risk factors and the Framingham risk score (adjusted odds ratio [OR]: 1.85; 95% CI: 1.15–2.98). However, the authors also reported that this ratio was no better than total cholesterol/HDL ratio for discriminating between cases and controls (AUC 0.673 vs. 0.670, respectively; p=0.38).

<u>Apo A-I as a Treatment Target</u>. A number of studies have evaluated the utility of the apo B/apo A-I ratio as a marker of treatment response in RCTs of statin treatment. Kastelein et al. (16)

combined data from 2 RCTs, the TNT and IDEAL trials, to compare the relationship between response to lipids, apo B/apo A-I ratio, and other lipid measures. This analysis included 18,889 patients with established coronary disease randomized to low- or high-dose statin treatment. In pairwise comparisons, the ratio of apo B/apo A-I was a significant predictor of events (HR: 1.24; 95% CI: 1.17-1.32) while the total/HDL cholesterol was not.

The PROVE-IT TIMI study (28) randomized 4,162 patients with acute coronary syndrome (ACS) to standard statin therapy or intensive statin therapy. While the on-treatment ratio of apo B/apo A-I ratio was a significant predictor of cardiac events (HR for each standard deviation [SD] increment: 1.10, 95% CI: 1.01–1.20), it was not superior to LDL-C (HR: 1.20, 95% CI: 1.07-1.35) or the total cholesterol/HDL ratio (HR: 1.12; 95% CI: 1.01–1.24) as a predictor of cardiac events.

Preliminary studies of infusions of reconstituted apo A-1 have demonstrated plaque regression in a small number of patients with acute coronary syndrome. (29) Based on this research, there is interest in developing synthetic apo A-1 mimetic proteins, and such agents are in the drug development stage. These types of agents would likely be targeted for patients with residual cardiac risk following maximal statin therapy, especially patients with low HDL levels.

<u>Conclusions</u>. The current evidence generally supports the contention that measurement of apo A-I, and the apo B/apo A-I ratio, is as good as or better than currently used lipid measures such as LDL and HDL. Some experts argue that the apo B/apo A-I ratio is superior to the LDL/HDL ratio as a predictor of cardiovascular risk and should supplement or replace traditional lipid measures as both a risk marker and a treatment target. (30, 31) However, there is substantial uncertainty regarding the degree of improvement that these measures provide. The evidence suggests that any incremental improvement in predictive ability over traditional measures is likely to be small and of uncertain clinical significance.

The use of apo A-I and the apo B/apo A-I ratio as a target of treatment response to statins may also be as good or better than the traditional measure of LDL. However, to improve outcomes, clinicians must have the tools to translate this information into clinical practice. Such tools for linking apo A-I to clinical decision making, both in risk assessment and treatment response, are currently not available. Apo A-I has not been incorporated into quantitative risk assessment models or treatment guidelines that can be used in clinical practice, such as the ATP III. (1) The ATP III practice guidelines continue to tie clinical decision making to conventional lipid measures, such as total cholesterol (TC), LDL-C, and HDL-C. Therefore, it is not yet possible to conclude that these measures improve outcomes or that they should be adopted in routine clinical care. There is continued interest in developing new therapeutic agents that raise HDL, and apo A-I mimetics are currently in development for this purpose.

Apolipoprotein E

<u>Apo E as a Predictor of Cardiovascular Disease</u>. A large body of research has established a correlation between lipid levels and the underlying apo E genotype. For example, in population studies, the presence of an apo e2 allele is associated with the lowest cholesterol levels and the apo e4 allele is associated with the highest levels. (32, 33)

Numerous studies have focused on the relationship between genotype and physiologic markers of atherosclerotic disease. A number of small- to medium-sized cross-sectional and case-control studies have correlated apo E with surrogate outcomes such as cholesterol levels, markers of inflammation, or carotid intima-media thickness. (34-39) These studies have generally shown a relationship between apo E and these surrogate outcomes. Other studies have suggested that carriers of apo e4 are more likely to develop signs of atherosclerosis independent of total and LDL-cholesterol levels. (40-43)

Some larger observational studies have correlated apo E genotype with clinical disease. The Atherosclerosis Risk in Communities (ARIC) study followed up 12,000 middle-aged individuals free of CAD at baseline for 10 years. (8) This study reported that the e3/2 genotype was associated with carotid artery atherosclerosis after controlling for other atherosclerotic risk factors. Volcik et al. reported that apo E polymorphisms were associated with LDL levels and carotid intima-media thickness but were not predictive of incident CAD. (44)

A meta-analysis published by Bennet and colleagues (45) summarized the evidence from 147 studies on the association of apo E genotypes with lipid levels and cardiac risk. Eighty-two studies included data on the association of apo E with lipid levels, and 121 studies reported the association with clinical outcomes. The authors estimated that patients with the apo e2 allele had LDL levels that were approximately 31% less compared to patients with the apo e4 allele. When compared to patients with the apo e3 allele, patients with apo e2 had an approximately 20% decreased risk for coronary events (OR: 0.80; 95% CI: 0.70–0.90). Patients with the apo e4 had an estimated 6% higher risk of coronary events that was of marginal statistical significance (OR: 1.06; 95% CI: 0.99–1.13).

<u>Apo E as a Predictor of Response to Therapy</u>. Apo E has been investigated as a predictor of response to therapy by examining apo E alleles in the intervention arm(s) of lipid-lowering trials. Some data suggest that patients with an apo e4 allele may respond better to diet-modification strategies. (46, 47) Other studies have suggested that response to statin therapy may vary with apo E genotype and that the e2 allele indicates greater responsiveness to statins. (46, 48)

Chiodini et al. (49) examined differential response to statin therapy according to apo E genotype, by reanalyzing data from the GISSI study according to apo E genotype. GISSI was an RCT comparing pravastatin with placebo in 3,304 Italian patients with previous myocardial infarction (MI). Patients with the apo e4 allele treated with statins had a greater response to treatment as evidenced by lower overall mortality (1.85% vs. 5.28%, respectively, p=0.023), while there was no difference in mortality for patients who were not treated with statins (2.81% vs. 3.67%, respectively, p=0.21). This study corroborates results reported in previous studies but does not provide evidence to suggest that changes in treatment should be made as a result of apo E genotype.

For the 2009 policy update, additional published studies were identified that evaluated apo E genetic status as a predictor of response to lipid-lowering therapy. Donnelly et al. (50) reported on 1,383 patients treated with statins from the Genetics of Diabetes Audit and Research in Tayside, Scotland (Go-DARTS) database. The researchers reported on the final LDL levels and percent of patients achieving target LDL according to apo E genetic status. LDL levels following treatment were lower for patients who were homozygous for apo e2, compared to patients homozygous for apo e4 (0.6 +/- 0.5 mmol/L vs. 1.7 +/- 0.3 mmol/L, p<0.001). All patients who were homozygous for apo e2 reached their target LDL level, compared to 68% of patients homozygous for apo e4 (p<0.001).

Vossen et al. (51) evaluated response to diet and statin therapy by apo e status in 981 patients with CAD who were enrolled in a cardiac rehabilitation program. These authors reported that patients with an apo e4 allele were more responsive to both diet and statin therapy than were patients with an apo e2 allele. The overall response to treatment was more dependent on baseline LDL levels than apo e genetic status, with 30–47% of the variation in response to treatment explained by baseline LDL, compared to only 1% of the variation explained by apo E status.

Conclusions. The evidence suggests that apo E genotype may be associated with lipid levels and CAD but is probably not useful in providing additional clinically relevant information beyond established risk factors. Apo E is considered a relatively poor predictor of CAD, especially when compared to other established and emerging clinical variables and does not explain a large percent of the inter-individual variation in total cholesterol (TC) and LDL levels. Moreover, apo E has not been incorporated into standardized cardiac risk assessment models and was not identified as one of the important "emerging risk factors" in the most recent ATP III recommendations.

The evidence on response to treatment indicates that apo E genotype may be a predictor of response to statins and may allow clinicians to better gauge an individual's chance of successful treatment, although not all studies are consistent in reporting this relationship. At present, it is unclear how this type of information will change clinical management. Dietary modifications are a universal recommendation for those with elevated cholesterol or LDL levels, and statin drugs are the overwhelmingly preferred agents for lipid-lowering therapy. It is unlikely that a clinician will choose alternative therapies, even in the presence of an apo E phenotype that indicates diminished response.

None of the available evidence provides adequate data to establish that apo E genotype or phenotype improves outcomes when used in clinical care. Thus, given the uncertain impact on clinical outcomes, this testing is considered investigational.

LDL subclass and LDL particle size/concentration

LDL Subclass as an Independent Risk Factor for Cardiovascular Disease. A nested case-control study from the Physician's Health Study, a prospective cohort study of approximately 15,000 men, investigated whether LDL particle size was an independent predictor of CAD risk, particularly in comparison to triglyceride levels. (52) This study concluded that while LDL particle diameter was associated with risk of MI, this association was not present after adjustment for triglyceride level. Only triglyceride level was significant independently.

The Quebec Cardiovascular Study (4, 53) evaluated the ability of "nontraditional" lipid risk factors, including LDL size, to predict subsequent CAD events in a prospective cohort study of 2,155 men followed up for 5 years. The presence of small LDL was associated with a 2.5 times increased risk for ischemic heart disease after adjustment for traditional lipid values, indicating a level of risk similar to total LDL. This study also suggested an interaction in atherogenic risk between LDL size and apolipoprotein B levels. In the presence of small LDL particles, elevated apolipoprotein B levels were associated with a 6-fold increased risk of CAD, whereas when small LDL particles were not present, elevated apolipoprotein B levels were associated with only a 2-fold increase in risk.

In 2005, Tzou and colleagues examined the clinical value of "advanced lipoprotein testing" in 311 randomly selected adults participating in the Bogalusa Heart Study. (54) Advanced lipoprotein testing consisted of subclass patterns of LDL, i.e., the presence of large buoyant particles, intermediate particles, or small dense particles. These measurements were used to predict the presence of subclinical atherosclerosis, as measured ultrasonographically by carotid intimal-media thickness. In multivariate logistic regression models, substituting advanced lipoprotein testing for corresponding traditional lipoprotein values did not improve prediction of the highest quartile of carotid intimal-media thickness.

LDL Subclass as a Predictor of Treatment Response. Patients with subclass pattern B have been reported to respond more favorably to diet therapy compared to those with subclass pattern A. (55) Subclass pattern B has also been shown to respond more favorably to the drugs gemfibrozil and niacin, with a shift from small, dense LDL particles to larger LDL particles. While statin drugs lower the overall concentration of LDL cholesterol, there is no shift to the larger LDL particles. (56)

Superko and colleagues (57) reported that the response to gemfibrozil differed in patients with LDL subclass A compared to those with LDL subclass B. There was a greater reduction in the small, low-density LDL levels for patients with subclass B, but this was not correlated with clinical outcomes. Another study reported that atorvastatin treatment led to an increase in mean LDL size, while pravastatin treatment led to a decrease in LDL size. (58)

These studies generally confirmed that small, dense LDL is impacted preferentially by fibrate treatment (59-61) and possibly also by statin therapy. (59, 61) However, none of the studies demonstrate that preferentially targeting small, dense LDL leads to improved outcomes, as compared to using the standard LDL targets that are widespread in clinical care.

Several trials with angiographic outcomes have examined the change in LDL particle size in relation to angiographic progression of CAD. The Stanford Coronary Risk Intervention Project (SCRIP) trial studied the relationship between small, dense LDL and the benefit of diet, counseling, and drug therapy in patients with CAD, as identified by initial coronary angiogram. (62) Patients with subclass pattern B showed a significantly greater reduction in CAD progression, compared to those with subclass pattern A. The Familial Atherosclerosis Treatment Study (FATS) randomized patients from families with premature CAD and elevated apolipoprotein B levels. (63) Change in LDL particle size was significantly correlated with angiographic progression of CAD in this study. Fewer studies have evaluated clinical outcomes in relation to LDL particle size. In the Cholesterol and Recurrent Events (CARE) trial, survivors of MI with normal cholesterol levels were randomly assigned to lipid-lowering therapy or placebo. A post hoc analysis from this trial failed to demonstrate a correlation between change in particle size and treatment benefit. (64)

Measurement of LDL Particle Size and Concentration by NMR. Similar to small dense lipoprotein particles, several epidemiologic studies have shown that the lipoprotein particle size and concentration measured by nuclear magnetic resonance (NMR) is also associated with cardiac risk. For example, the data derived from the Cardiovascular Health Study, Women's Health Study, and PLAC-1 trial suggest that the number of LDL particles is an independent predictor of cardiac risk. (65-67) Translating these findings into clinical practice requires setting target values for lipoprotein number. Proposed target values have been derived from the same data set (i.e., the Framingham study) that was used to set the ATP III target goals for LDL-C. For example, the

ATP III targets for LDL-C correspond to the 20th, 50th, and 80th percentile values in the Framingham Offspring Study, depending on the number of risk factors present. Proposed target goals for lipoprotein number correspond to the same percentile values, and LDL particle concentrations corresponding to the 20th, 50th, and 80th percentile are 1,100 nmol/L, 1,400 nmol/L, and 1,800 nmol/L, respectively. (68)

Following the publication of several clinical trials indicating benefit for intensive statin therapy with lowering of LDL goals beyond those recommended in the ATP III guidelines, (21) many experts recommend a further lowering of the target LDL-C from 100 mg/dL to 70 mg/dL in high-risk patients. These new, more aggressive targets of therapy create additional questions of how either measurements of either LDL concentration or LDL size can be used to improve patient management.

Mora et al. (69) evaluated the predictive ability of LDL particle size and number measured by NMR in participants of the Women's Health Study, a prospective cohort study of 27,673 women followed over an 11-year period. After controlling for nonlipid factors, LDL particle number was a significant predictor of incident cardiovascular disease, with a hazard ratio of 2.51 (95% CI: 1.91-3.30) for the highest, compared to the lowest quintile. LDL particle size was similarly predictive of cardiovascular risk, with a hazard ratio of 0.64 (95% CI: 0.52–0.79). When compared to standard lipid measures and apolipoproteins, LDL particle size and number showed similar predictive ability but were not superior in predicting cardiovascular events.

Current Treatment Guidelines. A 2008 consensus statement commented on the use of LDL particle number in patients with cardiometabolic risk. (22) This article comments on the limitations of the clinical utility of NMR measurement of LDL particle number or size, including lack of widespread availability. This article also comments that there is a need for more independent data confirming the accuracy of the method and whether its predictive power is consistent across various patient populations.

Conclusions. Small LDL size is one component of an atherogenic lipid profile that also includes increased triglycerides, increased apolipoprotein B, and decreased HDL. Some studies have reported that LDL size is an independent risk factor for CAD, and others have reported that a shift in LDL size may be a useful marker of treatment response. However, the direct clinical application of measuring small, dense lipoprotein particles is still unclear. To improve outcomes, clinicians must have the tools to translate this information into clinical practice. Such tools for linking levels of small, dense LDL to clinical decision making, both in risk assessment and treatment response, are currently not available. Published data are inadequate to determine how such measurements should guide treatment decisions and whether these treatment decisions result in beneficial patient outcomes.

A relatively small number of studies have evaluated the predictive ability of LDL particle size and number as measured by NMR. These studies do not demonstrate that NMR-measured particle size and/or number offer additional predictive ability beyond that provided by traditional lipid measures. NMR measures have been proposed as indicators of residual cardiovascular risk in patients treated with statins who have met LDL goals, but there is no evidence that these measures improve health outcomes when used for this purpose.

Based on the available evidence and the uncertain impact of testing on clinical outcomes, testing for LDL subclass and LDL particle size/concentration is considered investigational.

HDL subclass and HDL particle size/concentration

HDL subclass as a predictor of cardiovascular risk. A large number of prospective observational studies have examined the relationship between HDL subclass and cardiovascular risk. A representative sample of some of the most salient studies is discussed below.

In the Kuopio Ischemic Heart Disease Risk Factor Study, both total HDL-C and levels of HDL-2 had significant independent associations with risk of acute MI. (70) The Quebec Cardiovascular Study investigated the association of HDL-2 and HDL-3 subclasses with ischemic heart disease in a subsample of 944 French-Canadian men participating in the larger trial. (4) During the 10-year follow-up, levels of HDL-2 were statistically significant as independent predictors of CAD events, but the difference in predictive value with and without HDL subclasses was not considered clinically significant.

In contrast, some studies have not reported HDL subclassification to be an independent predictor of CAD. The ARIC study, a large prospective cohort study, followed 12,000 middle-aged individuals free of CAD at baseline for 10 years. (8) In this study, prediction of CAD was not improved by the addition of either apo A-I levels or HDL density. Similarly, in the Physicians' Health Study (71) and the Caerphilly and Speedwell Collaborative Heart Disease Studies, (72) both of which were studies of middle-aged men, risk prediction based on HDL-C was also not improved by HDL subclassification.

HDL subclass as treatment target. There were no clinical trials that evaluated the use of HDL subclass as treatment target.

Measurement of HDL Particle Size and Concentration by NMR. The relationship between HDL particle size and the risk of coronary heart disease was examined in the EPIC-Norfolk cohort study using a nested case-control design. (73) In the EPIC-Norfolk study, healthy individuals between the age of 45-79 years were enrolled and followed for the development of coronary disease. The nested case control study matched 1,035 individuals who developed coronary disease with 1,920 controls who did not develop coronary disease. Small particle size was associated with an adverse cardiometabolic risk profile, and an increased risk of coronary disease in men (OR: 1.43; 95% CI: 1.01-2.03) but not in women (OR: 0.84; 95% CI: 0.52-1.35).

In a post-hoc analysis from the EPIC-Norfolk study, El Harchaoui et al. (74) measured HDL particle size and number using NMR spectroscopy and gradient gel electrophoresis. The authors reported numerous multivariate regression models, controlling for various combinations of other lipid measures. HDL particle number was an independent predictor of CAD risk in all of the models reported. HDL particle size was an independent predictor in some models, but significance was lost when apo B was included as a covariate.

Conclusions. Numerous measures have been used in HDL subclass testing. The current evidence generally supports the contention that HDL subclass testing may add independent predictive information to standard lipid measurements. To improve outcomes, clinicians must have the tools to translate this information into clinical practice. Such tools for linking HDL subclasses to clinical decision making, both in risk assessment and treatment response, are currently not available.

HDL subclassification has not been incorporated into quantitative risk assessment models or treatment guidelines that can be used in clinical practice, such as the ATP III. The ATP III practice guidelines continue to tie clinical decision making to conventional lipid measures, such as total cholesterol, LDL-C, and HDL-C. Therefore, it is not yet possible to conclude that these measures improve outcomes or that they should be adopted in routine clinical care.

None of the available evidence is sufficient to demonstrate impact on clinical outcomes, thus testing for HDL subclass testing is considered investigational.

Lipoprotein A

Lipoprotein A as a Predictor of Cardiovascular Risk. Numerous prospective cohort studies and systematic reviews have evaluated lipoprotein (a) (lp[a]) as a cardiovascular risk factor. The following are representative prospective trials drawn from the extensive literature on this topic.

The Emerging Risk Factors Collaboration published a patient-level meta-analysis of 37 prospective cohort studies enrolling 154,544 individuals. (3) Risk prediction was examined for a variety of traditional and non-traditional lipid markers. For lp(a), evidence from 24 studies on 133,502 individuals reported that lp(a) was an independent risk factor for reduced cardiovascular risk, with an adjusted hazard ratio for cardiovascular events of 1.13 (95% CI: 1.09-1.18). The addition of lp(a) to traditional risk factors resulted in a small improvement in risk prediction, with an increase in the C-statistic of approximately 0.002. On reclassification analysis, there was no significant improvement in the net reclassification index (0.05%, 95% CI: -0.59 to 0.70).

A systematic review by Genser et al. (75) included 67 prospective studies on 181,683 individuals that evaluated the risk of cardiovascular disease associated with lp(a). Pooled analysis was performed on 37 studies that reported the endpoints of cardiovascular events. When grouped by design and populations, the relative risks for these studies, comparing the uppermost and lowest strata of lp(a), ranged from 1.64-2.37. The RR for cardiovascular events was higher in patients with previous cardiovascular disease compared to patients without previous disease. There were no significant associations found between lp(a) levels, overall mortality, or stroke.

The Lipid Research Clinics (LRC) Coronary Primary Prevention Trial, one of the first large-scale, RCTs of cholesterol-lowering therapy, measured initial lp(a) levels and reported that lp(a) was an independent risk factor for CAD when controlled for other lipid and non-lipid risk factors. (76) As part of the Framingham offspring study, lp(a) levels were measured in 2,191 asymptomatic men between the ages of 20 and 54 years. (77) After a mean follow-up of 15 years, there were 129 coronary heart disease events, including MI, coronary insufficiency, angina, or sudden cardiac death. Comparing the lp(a) levels of these patients with the other participants, the authors concluded that elevated lp(a) was an independent risk factor for the development of premature coronary heart disease (i.e., before age 55 years). The ARIC study evaluated the predictive ability of lp(a) in 12,000 middle-aged individuals free of CAD at baseline who were followed up for 10 years. (8) The lp(a) levels were significantly higher among patients who developed CAD compared with those who did not, and lp(a) levels were an independent predictor of CAD above traditional lipid measures.

Kamstrup and colleagues (78) analyzed data from the Copenhagen City Heart Study, which followed up 9,330 individuals from the Copenhagen general population over a period of 10 years. This study reported a graded increase in risk of cardiac events with increasing lp(a) levels. At extreme levels of lp(a) above the 95th percentile, the adjusted hazard ratio for MI was 3.6 (95% CI: 1.7–7.7) for women and 3.7 (95% CI: 1.7–8.0) in men. Tzoulaki and colleagues (79) reported data from the Edinburgh Artery Study, which was a population cohort study that followed up 1,592 individuals for a mean of 17 years. These authors reported that lp(a) was an independent predictor of MI, with an odds ratio of 1.49 (95% CI: 1.0–2.2) for the highest one-third versus the lowest one-third.

Zakai and co-workers (80) evaluated 13 potential biomarkers for independent predictive ability compared to established risk factors, using data from 4,510 individuals followed up for 9 years in the Cardiovascular Health Study. The lp(a) was 1 of 7 biomarkers that had incremental predictive ability above established risk factors. The adjusted hazard ratio for each standard deviation increase in lp(a) was 1.07 (95% CI: 1.0-1.12).

Some studies, however, have failed to demonstrate such a relationship. In the Physicians' Health Study, initial lp(a) levels in the 296 participants who subsequently experienced MI were compared with lp(a) levels in matched controls who remained free from CAD. (81) The authors found that the distribution of lp(a) levels between the groups was identical. The European Concerted Action on Thrombosis and Disabilities (ECAT) study, a trial of secondary prevention, evaluated lp(a) as a risk factor for coronary events in 2,800 patients with known angina pectoris. (82) In this study, lp(a) levels were not significantly different among patients who did and did not have subsequent events, suggesting that lp(a) levels were not useful risk markers in this population.

Some researchers have hypothesized that there is a stronger relationship between lp(a) and stroke than for coronary heart disease. Similar to the situation with cardiac disease, the majority of prospective studies, but not all, have indicated that lp(a) is an independent risk factor for stroke. In 1 prospective cohort study, Rigal and co-workers (83) reported that an elevated lp(a) level was an independent predictor of ischemic stroke in men (OR: 3.55; 95% CI: 1.33–9.48) but not in women (OR: 0.42; 95% CI: 0.12–1.26). In the ARIC prospective cohort study of 14,221 participants, (84) elevated lp(a) was a significant independent predictor of stroke in African-American women (RR: 1.84; 95% CI: 1.05-3.07) and white women (RR: 2.42; 95% CI: 1.30–4.53) but not in African-American men (RR: 1.72; 95% CI: 0.86–3.48) or white men (RR: 1.18; 95% CI: 0.47–2.90).

There also may be a relationship between lp(a) as a cardiovascular risk factor and hormone status in women. Suk Danik et al. (85) reported the risk of a first cardiovascular event over a 10-year period in 27,736 women enrolled in the Women's Health Study. After controlling for standard cardiovascular risk factors, lp(a) was an independent predictor of risk in women who were not taking hormonal replacement therapy (HR: 1.77; 95% CI: 1.36–2.30, $p<0.0001$). However, for women who were taking hormonal replacement therapy, lp(a) levels were not a significant independent predictor of cardiovascular risk (HR: 1.13; 95% CI: 0.84–1.53, $p=0.18$).

Several meta-analyses have also examined the relationship between lp(a) levels and cardiovascular risk. Bennet et al. (86) synthesized the results of 31 prospective studies with at least 1 year of follow-up and that reported data on cardiovascular death and nonfatal MI. The combined results revealed a significant positive relationship between lp(a) and cardiovascular risk, with an odds ratio for patients with lp(a) in the top-third compared to those in the bottom-third of 1.45 (95% CI: 1.32–1.58). This analysis reported a moderately high degree of heterogeneity in the included studies (I2=43%), reflecting the fact that not all studies reported a significant positive association.

Smolders et al. summarized evidence from observational studies on the relationship between lp(a) and stroke. (87) Five prospective cohort studies and 23 case-control studies were included in this meta-analysis. Results from prospective cohort studies, lp(a) added a modest amount of incremental predictive information (combined RR for the highest one-third of lp(a): 1.22; 95% CI: 1.04–1.43). From case-control studies, an elevated lp(a) level was also associated with an increased risk of stroke (combined OR 2.39; 95% CI: 1.57–3.63).

A patient-level meta-analysis of 36 prospective studies published between 1970 and 2009 included 126,634 participants. (88) Overall, the independent association of lp(a) with vascular disease was consistent across studies but modest in size. The combined risk ratio, adjusted for age, sex, and traditional lipid risk factor, was 1.13 (95% CI: 1.09–1.18) for coronary heart disease and 1.10 (95% CI: 1.02–1.18) for ischemic stroke. There was no association of lp(a) levels with mortality.

Genetic studies have examined the association of various genetic loci with lp(a) levels, and Mendelian randomization studies have examined whether lp(a) is likely to be causative for CAD. In one such study, (89) there were 3 separate loci identified for increased lp(a) levels. Genetic variants were identified at 2 of these loci that were independently associated with coronary disease (OR: 1.70; 95% CI: 1.49–1.95, and OR: 1.92; 95% CI: 1.48–2.49). This finding strongly implies that elevated lp(a) levels are causative of coronary disease, as opposed to simply being associated.

<u>Lipoprotein A as Treatment Target</u>. There is a lack of evidence to determine whether lp(a) can be used as a target of treatment. Several randomized studies of lipid-lowering therapy have included measurements of lp(a) as an intermediate outcome measurement. While these studies have demonstrated that lp(a) levels are reduced in patients receiving statin therapy, the data are inadequate to demonstrate how this laboratory test can be used to improve patient management. (90, 91)

<u>Conclusions</u>. A large amount of epidemiologic evidence has determined that lp(a) is an independent risk factor for cardiovascular disease. The overall degree of risk associated with lp(a) levels appears to be modest, and the degree of risk may be mediated by other factors such as LDL levels and/or hormonal status. There is considerable uncertainty regarding the clinical utility of measuring lp(a), specifically how knowledge of lp(a) levels can be used in clinical care of patients who are being evaluated for lipid disorders. There is scant evidence on the use of lp(a) as a treatment target for patients with hyperlipidemia. The available evidence is insufficient related to impact on clinical outcomes; testing for lp(a) is considered investigational.

Summary

Numerous non-traditional lipid measurements have been proposed for use in improving risk prediction for cardiovascular disease, including apo B, apo A-1, the ratio of apo B/apo A-1, apo E, lipoprotein A, and subclasses of LDL and HDL. In general, there is evidence that these markers provide some incremental accuracy in risk prediction. However, it has not been established that the incremental accuracy provides clinically important information beyond that of traditional lipid measures. Furthermore, no study has provided high-quality evidence that measurement of markers leads to changes in management that improve health outcomes.

Some markers, e.g. apo B, have also been proposed as treatment targets for lipid-lowering therapy. While some evidence supports that they may be accurate in predicting residual risk for patients on lipid-lowering therapy, there is no high-quality evidence that these markers lead to health outcome improvements when used in place of traditional lipid targets, such as LDL. Because of the deficiencies in the literature around these issues, the use of these novel lipid risk markers remains investigational.

Practice Guidelines and Position Statements

The American College of Cardiology/American Heart Association published guidelines in 2010 for the assessment of cardiovascular risk in asymptomatic patients. (92) These guidelines included recommendations on measurement of some non-traditional lipid and apolipoproteins in cardiovascular risk assessment. Apo B, apo A, the ratio of apo B/apo A, lipoprotein a, lipid particle size, and lipid density were specifically addressed. Measurement of these specific lipid parameters were not recommended in asymptomatic individuals (level of evidence C).

The USPSTF issued recommendations in 2009 (USPSTF 2009) (93) on the use of nontraditional risk factors for the assessment of coronary heart disease. They included lipoprotein (a) in their summary statement that stated “The evidence is insufficient to assess the balance of benefits and harms of using the nontraditional risk factors discussed in this statement to screen asymptomatic men and women with no history of CHD to prevent CHD events”.

The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III) issued a position statement in 2001. (1) Apo B, apo A-1, lipid subclass, and lipoprotein (a) were listed as “emerging risk factors” for cardiovascular risk assessment, without specific recommendations for how these measures should be used in clinical practice.

A publication from a consensus conference of the American Diabetes Association and the American College of Cardiology Foundation (22) included specific recommendations for incorporating apo B testing into clinical care for high-risk patients. They recommended that for patients with metabolic syndrome who are being treated with statins, both LDL-C and apo B should be used as treatment targets, with an apo B target of less than 90 mg/dL. This consensus statement also commented on the use of LDL particle number in patients with cardiometabolic risk. They commented on the limitations of the clinical utility of NMR measurement of LDL particle number or size, including lack of widespread availability. They also mentioned that there is a need for more independent data confirming the accuracy of the method and whether its predictive power is consistent across various patient populations.

A Canadian task force has also endorsed use of apo B as a treatment target and proposed a target apo B level of 90 mg/dL. (23) These guidelines also recommended that a lipoprotein (a) concentration greater than 30 mg/dL with elevated LDL or other major risk factors may indicate the need for earlier and more intensive therapy to lower the LDL-C level.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

CPT/HCPCS

82172	Apolipoprotein, each
82664	Electrophoretic technique, not elsewhere specified
83695	Lipoprotein (a)
83700	Lipoprotein, blood; electrophoretic separation and quantitation
83701	Lipoprotein, blood; high resolution fractionation and quantitation of lipoproteins including lipoprotein subclasses when performed (eg, electrophoresis, ultracentrifugation)
83704	Lipoprotein, blood; quantitation of lipoprotein particle numbers and lipoprotein particle subclasses (eg, by nuclear magnetic resonance spectroscopy)
84181	Protein; Western Blot, with interpretation and report, blood or other body fluid
84999	Unlisted chemistry procedure

- There is no specific CPT code for measurement of apolipoprotein B. CPT code 82172 might be used.
- There is no specific code for apo E phenotyping or genotyping. For phenotyping, CPT code 84181 may be used.
- There is no CPT code for subclassification that is specific to high-density lipoprotein (HDL). CPT code 82664 or 83701 may be used.
- There is a CPT code for lipoprotein particle number and subclass quantification by nuclear magnetic resonance spectroscopy that is also not specific to HDL which is 83704.
- There is a specific CPT code for lipoprotein (a) testing which is 83695.
- Effective in 2006, 83716 was replaced with new CPT codes 83700, 83701, and 83704.

DIAGNOSES

Experimental / investigational for all diagnoses related to this policy.

REVISIONS

02-10-2011	▪ Created a new medical policy entitled Novel Lipid Risk Factors in Risk Assessment and Management of Cardiovascular Disease which replaced the following medical policies: 1. Apolipoprotein B in the Risk Assessment and Management of Cardiovascular Disease 2. Apolipoprotein E Genotype or Phenotype in the Management of Cardiovascular Disease 3. High-Density Lipoprotein Subclass Testing in the Diagnosis and Management of Cardiovascular Disease 4. Lipoprotein(a) Enzyme Immunoassay in the Management of Cardiovascular Disease

	5. Small Low-Density Lipoprotein (LDL) Particles and concentration of LDL Particles in Cardiac Risk Assessment and Management ▪ No policy language changes were made. Services in the previous medical policies were considered experimental / investigational and continue to be experimental / investigational in the new policy.
09-20-2011	Description section updated.
	Rationale section added.
	References section updated.
09-18-2012	Description section updated.
	Rationale section added.
	References section updated.

REFERENCES

1. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001; 285(19):2486-97.
2. BlueCross and BlueShield Technology Evaluation Center (TEC). C-Reactive Protein as a Cardiac Risk Marker (Special Report). TEC Assessment 2002; Volume 17, Tab 23.
3. Di Angelantonio E GP, Pennells L, et al. Lipid-Related Markers and Cardiovascular Disease Prevention. JAMA 2012; 307(23):2499-506.
4. Lamarche B, Moorjani S, Lupien PJ et al. Apolipoprotein A-I and B levels and the risk of ischemic heart disease during a five-year follow-up of men in the Quebec cardiovascular study. Circulation 1996; 94(3):273-8.
5. Walldius G, Jungner I, Holme I et al. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. Lancet 2001; 358(9298):2026-33.
6. Ridker PM, Rifai N, Cook NR et al. Non-HDL cholesterol, apolipoproteins A-I and B100, standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women. JAMA 2005; 294(3):326-33.
7. Benn M, Nordestgaard BG, Jensen GB et al. Improving prediction of ischemic cardiovascular disease in the general population using apolipoprotein B: the Copenhagen City Heart Study. Arterioscler Thromb Vasc Biol 2007; 27(3):661-70.
8. Sharrett AR, Ballantyne CM, Coady SA et al. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation 2001; 104(10):1108-13.
9. Rasouli M, Kiasari AM, Mokhberi V. The ratio of apoB/apoAI, apoB and lipoprotein(a) are the best predictors of stable coronary artery disease. Clin Chem Lab Med 2006; 44(8):1015-21.
10. Walldius G, Jungner I. Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. J Intern Med 2004; 255(2):188-205.
11. Kappelle PJ, Gansevoort RT, Hillege JL et al. Apolipoprotein B/A-I and total cholesterol/high-density lipoprotein cholesterol ratios both predict cardiovascular events in the general population independently of nonlipid risk factors, albuminuria and C-reactive protein. J Intern Med 2011; 269(2):232-42.
12. Ridker PM, Buring JE, Rifai N et al. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. JAMA 2007; 297(6):611-9.

13. Ingelsson E, Schaefer EJ, Contois JH et al. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. JAMA 2007; 298(7):776-85.
14. Gotto AM, Jr., Whitney E, Stein EA et al. Relation between baseline and on-treatment lipid parameters and first acute major coronary events in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). Circulation 2000; 101(5):477-84.
15. Simes RJ, Marschner IC, Hunt D et al. Relationship between lipid levels and clinical outcomes in the Long-term Intervention with Pravastatin in Ischemic Disease (LIPID) Trial: to what extent is the reduction in coronary events with pravastatin explained by on-study lipid levels? Circulation 2002; 105(10):1162-9.
16. Kastelein JJ, van der Steeg WA, Holme I et al. Lipids, apolipoproteins, and their ratios in relation to cardiovascular events with statin treatment. Circulation 2008; 117(23):3002-9.
17. Mora S, Wenger NK, Demicco DA et al. Determinants of residual risk in secondary prevention patients treated with high- versus low-dose statin therapy: the Treating to New Targets (TNT) study. Circulation 2012; 125(16):1979-87.
18. Mora S, Glynn RJ, Boekholdt SM et al. On-treatment non-high-density lipoprotein cholesterol, apolipoprotein B, triglycerides, and lipid ratios in relation to residual vascular risk after treatment with potent statin therapy: JUPITER (justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin). J Am Coll Cardiol 2012; 59(17):1521-8.
19. Boekholdt SM, Arsenault BJ, Mora S et al. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. JAMA 2012; 307(12):1302-9.
20. Snow V, Aronson MD, Hornbake ER et al. Lipid control in the management of type 2 diabetes mellitus: a clinical practice guideline from the American College of Physicians. Ann Intern Med 2004; 140(8):644-9.
21. Grundy SM, Cleeman JI, Merz CN et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation 2004; 110(2):227-39.
22. Brunzell JD, Davidson M, Furberg CD et al. Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. Diabetes Care 2008; 31(4):811-22.
23. Genest J, Frohlich J, Fodor G et al. Recommendations for the management of dyslipidemia and the prevention of cardiovascular disease: summary of the 2003 update. CMAJ 2003; 169(9):921-4.
24. Barter PJ, Ballantyne CM, Carmena R et al. Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the thirty-person/ten-country panel. J Intern Med 2006; 259(3):247-58.
25. Clarke R, Emberson JR, Parish S et al. Cholesterol fractions and apolipoproteins as risk factors for heart disease mortality in older men. Arch Intern Med 2007; 167(13):1373-8.
26. Birjmohun RS, Dallinga-Thie GM, Kuivenhoven JA et al. Apolipoprotein A-II is inversely associated with risk of future coronary artery disease. Circulation 2007; 116(18):2029-35.
27. van der Steeg WA, Boekholdt SM, Stein EA et al. Role of the apolipoprotein B-apolipoprotein A-I ratio in cardiovascular risk assessment: a case-control analysis in EPIC-Norfolk. Ann Intern Med 2007; 146(9):640-8.
28. Ray KK, Cannon CP, Cairns R et al. Prognostic utility of apoB/AI, total cholesterol/HDL, non-HDL cholesterol, or hs-CRP as predictors of clinical risk in patients receiving statin therapy after acute coronary syndromes: results from PROVE IT-TIMI 22. Arterioscler Thromb Vasc Biol 2009; 29(3):424-30.

29. Osei-Hwedieh DO, Amar M, Sviridov D et al. Apolipoprotein mimetic peptides: Mechanisms of action as anti-atherogenic agents. Pharmacol Ther 2011; 130(1):83-91.
30. Mudd JO, Borlaug BA, Johnston PV et al. Beyond low-density lipoprotein cholesterol: defining the role of low-density lipoprotein heterogeneity in coronary artery disease. J Am Coll Cardiol 2007; 50(18):1735-41.
31. Sniderman AD, Kiss RS. The strengths and limitations of the apoB/apoA-I ratio to predict the risk of vascular disease: a Hegelian analysis. Curr Atheroscler Rep 2007; 9(4):261-5.
32. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988; 8(1):1-21.
33. Hallman DM, Boerwinkle E, Saha N et al. The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet 1991; 49(2):338-49.
34. Koch W, Hoppmann P, Schomig A et al. Apolipoprotein E gene epsilon2/epsilon3/epsilon4 polymorphism and myocardial infarction: case-control study in a large population sample. Int J Cardiol 2008; 125(1):116-7.
35. Kulminski AM, Ukraintseva SV, Arbeev KG et al. Health-protective and adverse effects of the apolipoprotein E epsilon2 allele in older men. J Am Geriatr Soc 2008; 56(3):478-83.
36. Schmitz F, Mevissen V, Krantz C et al. Robust association of the APOE epsilon4 allele with premature myocardial infarction especially in patients without hypercholesterolaemia: the Aachen study. Eur J Clin Invest 2007; 37(2):106-8.
37. Vaisi-Raygani A, Rahimi Z, Nomani H et al. The presence of apolipoprotein epsilon4 and epsilon2 alleles augments the risk of coronary artery disease in type 2 diabetic patients. Clin Biochem 2007; 40(15):1150-6.
38. Ciftdogan DY, Coskun S, Ulman C et al. The association of apolipoprotein E polymorphism and lipid levels in children with a family history of premature coronary artery disease. J Clin Lipidol 2012; 6(1):81-7.
39. Vasunilashorn S, Glei DA, Lan CY et al. Apolipoprotein E is associated with blood lipids and inflammation in Taiwanese older adults. Atherosclerosis 2011; 219(1):349-54.
40. de Andrade M, Thandi I, Brown S et al. Relationship of the apolipoprotein E polymorphism with carotid artery atherosclerosis. Am J Hum Genet 1995; 56(6):1379-90.
41. Eichner JE, Kuller LH, Orchard TJ et al. Relation of apolipoprotein E phenotype to myocardial infarction and mortality from coronary artery disease. Am J Cardiol 1993; 71(2):160-5.
42. Wilson PW, Myers RH, Larson MG et al. Apolipoprotein E alleles, dyslipidemia, and coronary heart disease. The Framingham Offspring Study. JAMA 1994; 272(21):1666-71.
43. Wilson PW, Schaefer EJ, Larson MG et al. Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. Arterioscler Thromb Vasc Biol 1996; 16(10):1250-5.
44. Volcik KA, Barkley RA, Hutchinson RG et al. Apolipoprotein E polymorphisms predict low density lipoprotein cholesterol levels and carotid artery wall thickness but not incident coronary heart disease in 12,491 ARIC study participants. Am J Epidemiol 2006; 164(4):342-8.
45. Bennet AM, Di Angelantonio E, Ye Z et al. Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA 2007; 298(11):1300-11.
46. Ordovas JM, Mooser V. The APOE locus and the pharmacogenetics of lipid response. Curr Opin Lipidol 2002; 13(2):113-7.
47. Sarkkinen E, Korhonen M, Erkkila A et al. Effect of apolipoprotein E polymorphism on serum lipid response to the separate modification of dietary fat and dietary cholesterol. Am J Clin Nutr 1998; 68(6):1215-22.

48. Carmena R, Roederer G, Mailloux H et al. The response to lovastatin treatment in patients with heterozygous familial hypercholesterolemia is modulated by apolipoprotein E polymorphism. Metabolism 1993; 42(7):895-901.
49. Chiodini BD, Franzosi MG, Barlera S et al. Apolipoprotein E polymorphisms influence effect of pravastatin on survival after myocardial infarction in a Mediterranean population: the GISSI-Prevenzione study. Eur Heart J 2007; 28(16):1977-83.
50. Donnelly LA, Palmer CN, Whitley AL et al. Apolipoprotein E genotypes are associated with lipid-lowering responses to statin treatment in diabetes: a Go-DARTS study. Pharmacogenet Genomics 2008; 18(4):279-87.
51. Vossen CY, Hoffmann MM, Hahmann H et al. Effect of APOE genotype on lipid levels in patients with coronary heart disease during a 3-week inpatient rehabilitation program. Clin Pharmacol Ther 2008; 84(2):222-7.
52. Stampfer MJ, Krauss RM, Ma J et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 1996; 276(11):882-8.
53. Lamarche B, Tchernof A, Moorjani S et al. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation 1997; 95(1):69-75.
54. Tzou WS, Douglas PS, Srinivasan SR et al. Advanced lipoprotein testing does not improve identification of subclinical atherosclerosis in young adults: the Bogalusa Heart Study. Ann Intern Med 2005; 142(9):742-50.
55. Kwiterovich PO, Jr. Clinical relevance of the biochemical, metabolic, and genetic factors that influence low-density lipoprotein heterogeneity. Am J Cardiol 2002; 90(8A):30i-47i.
56. Superko HR. Beyond LDL cholesterol reduction. Circulation 1996; 94(10):2351-4.
57. Superko HR, Berneis KK, Williams PT et al. Gemfibrozil reduces small low-density lipoprotein more in normolipemic subjects classified as low-density lipoprotein pattern B compared with pattern A. Am J Cardiol 2005; 96(9):1266-72.
58. Sirtori CR, Calabresi L, Pisciotta L et al. Effect of statins on LDL particle size in patients with familial combined hyperlipidemia: a comparison between atorvastatin and pravastatin. Nutr Metab Cardiovasc Dis 2005; 15(1):47-55.
59. Arca M, Montali A, Pigna G et al. Comparison of atorvastatin versus fenofibrate in reaching lipid targets and influencing biomarkers of endothelial damage in patients with familial combined hyperlipidemia. Metabolism 2007; 56(11):1534-41.
60. Rosenson RS, Wolff DA, Huskin AL et al. Fenofibrate therapy ameliorates fasting and postprandial lipoproteinemia, oxidative stress, and the inflammatory response in subjects with hypertriglyceridemia and the metabolic syndrome. Diabetes Care 2007; 30(8):1945-51.
61. Tokuno A, Hirano T, Hayashi T et al. The effects of statin and fibrate on lowering small dense LDL- cholesterol in hyperlipidemic patients with type 2 diabetes. J Atheroscler Thromb 2007; 14(3):128-32.
62. Miller BD, Alderman EL, Haskell WL et al. Predominance of dense low-density lipoprotein particles predicts angiographic benefit of therapy in the Stanford Coronary Risk Intervention Project. Circulation 1996; 94(9):2146-53.
63. Brown G, Albers JJ, Fisher LD et al. Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. N Engl J Med 1990; 323(19):1289-98.
64. Campos H, Moye LA, Glasser SP et al. Low-density lipoprotein size, pravastatin treatment, and coronary events. JAMA 2001; 286(12):1468-74.

65. Blake GJ, Otvos JD, Rifai N et al. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation 2002; 106(15):1930-7.
66. Kuller L, Arnold A, Tracy R et al. Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the cardiovascular health study. Arterioscler Thromb Vasc Biol 2002; 22(7):1175-80.
67. Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial. Am J Cardiol 2002; 90(2):89-94.
68. Otvos JD, Jeyarajah EJ, Cromwell WC. Measurement issues related to lipoprotein heterogeneity. Am J Cardiol 2002; 90(8A):22i-29i.
69. Mora S, Otvos JD, Rifai N et al. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. Circulation 2009; 119(7):931-9.
70. Salonen JT, Salonen R, Seppanen K et al. HDL, HDL2, and HDL3 subfractions, and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men. Circulation 1991; 84(1):129-39.
71. Stampfer MJ, Sacks FM, Salvini S et al. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. N Engl J Med 1991; 325(6):373-81.
72. Sweetnam PM, Bolton CH, Yarnell JW et al. Associations of the HDL2 and HDL3 cholesterol subfractions with the development of ischemic heart disease in British men. The Caerphilly and Speedwell Collaborative Heart Disease Studies. Circulation 1994; 90(2):769-74.
73. Arsenault BJ, Lemieux I, Despres JP et al. HDL particle size and the risk of coronary heart disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. Atherosclerosis 2009; 206(1):276-81.
74. El Harchaoui K, Arsenault BJ, Franssen R et al. High-density lipoprotein particle size and concentration and coronary risk. Ann Intern Med 2009; 150(2):84-93.
75. Genser B, Dias KC, Siekmeier R et al. Lipoprotein (a) and risk of cardiovascular disease--a systematic review and meta analysis of prospective studies. Clin Lab 2011; 57(3-4):143-56.
76. Schaefer EJ, Lamon-Fava S, Jenner JL et al. Lipoprotein(a) levels and risk of coronary heart disease in men. The Lipid Research Clinics Coronary Primary Prevention Trial. JAMA 1994; 271(13):999-1003.
77. Bostom AG, Cupples LA, Jenner JL et al. Elevated plasma lipoprotein(a) and coronary heart disease in men aged 55 years and younger. A prospective study. JAMA 1996; 276(7):544-8.
78. Kamstrup PR, Benn M, Tybjaerg-Hansen A et al. Extreme lipoprotein(a) levels and risk of myocardial infarction in the general population: the Copenhagen City Heart Study. Circulation 2008; 117(2):176-84.
79. Tzoulaki I, Murray GD, Lee AJ et al. Relative value of inflammatory, hemostatic, and rheological factors for incident myocardial infarction and stroke: the Edinburgh Artery Study. Circulation 2007; 115(16):2119-27.
80. Zakai NA, Katz R, Jenny NS et al. Inflammation and hemostasis biomarkers and cardiovascular risk in the elderly: the Cardiovascular Health Study. J Thromb Haemost 2007; 5(6):1128-35.
81. Ridker PM, Hennekens CH, Stampfer MJ. A prospective study of lipoprotein(a) and the risk of myocardial infarction. JAMA 1993; 270(18):2195-9.

82. Bolibar I, von Eckardstein A, Assmann G et al. Short-term prognostic value of lipid measurements in patients with angina pectoris. The ECAT Angina Pectoris Study Group: European Concerted Action on Thrombosis and Disabilities. Thromb Haemost 2000; 84(6):955-60.
83. Rigal M, Ruidavets JB, Viguier A et al. Lipoprotein (a) and risk of ischemic stroke in young adults. J Neurol Sci 2007; 252(1):39-44.
84. Ohira T, Schreiner PJ, Morrisett JD et al. Lipoprotein(a) and incident ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) study. Stroke 2006; 37(6):1407-12.
85. Suk Danik J, Rifai N, Buring JE et al. Lipoprotein(a), hormone replacement therapy, and risk of future cardiovascular events. J Am Coll Cardiol 2008; 52(2):124-31.
86. Bennet A, Di Angelantonio E, Erqou S et al. Lipoprotein(a) levels and risk of future coronary heart disease: large-scale prospective data. Arch Intern Med 2008; 168(6):598-608.
87. Smolders B, Lemmens R, Thijs V. Lipoprotein (a) and stroke: a meta-analysis of observational studies. Stroke 2007; 38(6):1959-66.
88. Erqou S, Kaptoge S, Perry PL et al. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. JAMA 2009; 302(4):412-23.
89. Clarke R, Peden JF, Hopewell JC et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. N Engl J Med 2009; 361(26):2518-28.
90. Bays HE, Dujovne CA, McGovern ME et al. Comparison of once-daily, niacin extended-release/lovastatin with standard doses of atorvastatin and simvastatin (the ADvicor Versus Other Cholesterol-Modulating Agents Trial Evaluation [ADVOCATE]). Am J Cardiol 2003; 91(6):667-72.
91. van Wissen S, Smilde TJ, Trip MD et al. Long term statin treatment reduces lipoprotein(a) concentrations in heterozygous familial hypercholesterolaemia. Heart 2003; 89(8):893-6.
92. Greenland P, Alpert JS, Beller GA et al. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2010; 56(25):e50-103.
93. Helfand M, Buckley DI, Freeman M et al. Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the U.S. Preventive Services Task Force. Ann Intern Med 2009; 151(7):496-507.