For dates of service 12/1/2016 and after this policy is no longer effective

BlueCross BlueShield of Alabama

Name of Blue Advantage Policy:

Novel Biomarkers in Risk Assessment and Management of Cardiovascular Disease

Policy #: 567
Category: Laboratory

Latest Review Date: December 2016
Policy Grade: B

Background:

Blue Advantage *medical policy does not conflict with Local Coverage Determinations (LCDs), Local Medical Review Policies (LMRPs) or National Coverage Determinations (NCDs) or with coverage provisions in Medicare manuals, instructions or operational policy letters. In order to be covered by Blue Advantage the service shall be reasonable and necessary under Title XVIII of the Social Security Act, Section 1862(a)(1)(A). The service is considered reasonable and necessary if it is determined that the service is:*

1. *Safe and effective;*
2. *Not experimental or investigational*;*
3. *Appropriate, including duration and frequency that is considered appropriate for the service, in terms of whether it is:*

    - *Furnished in accordance with accepted standards of medical practice for the diagnosis or treatment of the patient's condition or to improve the function of a malformed body member;*
    - *Furnished in a setting appropriate to the patient's medical needs and condition;*
    - *Ordered and furnished by qualified personnel;*
    - *One that meets, but does not exceed, the patient's medical need; and*
    - *At least as beneficial as an existing and available medically appropriate alternative.*

Routine costs of qualifying clinical trial services with dates of service on or after September 19, 2000 which meet the requirements of the Clinical Trials NCD are considered reasonable and necessary by Medicare. Providers should bill* ***Original Medicare* *for covered services that are related to* ***clinical trials*** *that meet Medicare requirements (Refer to Medicare National Coverage Determinations Manual, Chapter 1, Section 310 and Medicare Claims Processing Manual Chapter 32, Sections 69.0-69.11).*

Description of Procedure or Service:

Numerous lipid and nonlipid biomarkers have been proposed as potential risk markers for cardiovascular disease. The biomarkers assessed here are those that have the most evidence in support of their use in clinical care. The biomarkers assessed here are apolipoprotein B, apolipoprotein A-1, apolipoprotein E, B-type natriuretic peptide, cystatin C, fibrinogen, high-density lipoprotein (HDL) subclass, leptin, low-density lipoprotein (LDL) subclass, and lipoprotein A. These biomarkers have been studied as an alternative or addition to standard lipid panels for risk stratification in cardiovascular disease or as treatment targets for lipid-lowering therapy.

Low-density lipoproteins (LDL's) 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.

Other nonlipid markers have been identified as having an association with cardiovascular disease including B-type natriuretic peptide, cystatin C, fibrinogen and leptin. These biomarkers may have a predictive role in identifying cardiovascular disease risk or in targeting for therapy,

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 to measure LDL particle concentration. Particle size can be determined by gradient gel electrophoresis, or the number of LDL particles can be measured using nuclear magnetic resonance (NMR) spectroscopy. 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 AI

HDL contains two associated apolipoproteins, i.e., AI and AII. HDL particles can also be classified by whether they contain apolipoprotein AI (apo AI) only or whether they contain both apo AI and apolipoprotein AII (apo AII). All lipoproteins contain apo AI, and some also contain apo AII. Since all HDL particles contain apo AI, 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 AI 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 AI 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 three alleles (e2, e3, and e4) that code for three 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.

Brain Natriuretic Peptide (BNP)
BNP is an amino acid polypeptide that is secreted primarily by the ventricles of the heart when pressure to the cardiac muscles increases or there is myocardial ischemia. Elevations in BNP levels reflect deterioration in cardiac loading levels and may predict adverse events. BNP has been studied as a biomarker for managing heart failure and predicting cardiovascular and heart failure risk.

Cystatin C
Cystatin C is a small serine protease inhibitor protein that is secreted from all functional cells found throughout the body. It has primarily been used as a biomarker of kidney function. Cystatin C has also been studied to determine whether it may serve as a biomarker for predicting cardiovascular risk. Cystatin C is encoded by the *CST3* gene.

Fibrinogen
Fibrinogen is a circulating clotting factor and precursor of fibrin. It is important in platelet aggregation and a determinant of blood viscosity. Fibrinogen levels have been shown to be associated with future risk of cardiovascular risk and all-cause mortality.

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 $HDL_{2,}$ the larger, less dense particles that may have the greatest degree of cardioprotection, and HDL_3, which are smaller, denser particles. 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 (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 HDL_2 and HDL_3, 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.

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 two 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 to measure LDL particle concentration: the surrogate measurement of apo B or direct measurement of the number of particles using NMR. NMR signals distinguish lipoprotein subclasses of different size. Thus NMR can directly measure the number of LDL particles of a specific size (i.e., small dense LDL) and can measure the total number of particles. Thus, NMR is proposed as an additional technique to assess cardiac risk.

Leptin
Leptin is a protein secreted by fat cells that has been found to be elevated in heart disease. Leptin has been studied to determine if it has any relationship with the development of cardiovascular disease.

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.

For testing performed as a panel, see medical policy #538-Cardiovascular Risk Panels.

Policy:

Beginning December 1, 2016 this policy is no longer effective.

Effective for dates of service on or after January 9, 2015 and prior to December 1, 2016:
Blue Advantage will treat **measurement of novel lipid and nonlipid risk factors** (i.e., apolipoprotein B, apolipoprotein A-I, apolipoprotein E, B-type natriuretic peptide, cystatin C, fibrinogen, leptin, LDL subclass, HDL subclass, lipoprotein[a]) as a **non-covered benefit** and as **investigational** as an adjunct to LDL cholesterol in the risk assessment and management of cardiovascular disease. *

*based on 2013 ACC/AHA Guidelines for the Assessment of Cardiovascular Risk (see Key Points)

Effective for dates of service prior to January 9, 2015:
Blue Advantage will treat **measurement of small low density lipoprotein (LDL) particles, lipoprotein (a) [lp(a)] enzyme immunoassay and apolipoprotein B (apo B)** as a **as a covered benefit** when the patient has documented clinical coronary heart disease (CAD), diabetes mellitus, hyperlipidemia, peripheral arterial disease**,** abdominal aortic aneurysm, symptomatic carotid artery disease and/or first degree family member with history of early (males before age 55 and females before age 65) cardiovascular event.

Blue Advantage will treat **measurement of small low density Lipoprotein (LDL) particles, lipoprotein (a) [lp(a)] enzyme immunoassay and apolipoprotein B (apo B)** as a **non-covered benefit** when performed for screening or first line testing and is considered **investigational**.

Blue Advantage will treat **high-density lipoprotein subclass testing** for the screening, evaluation, and management of patients for cardiovascular disease as a **non-covered benefit** and as **investigational**.

Blue Advantage will treat **determination of the apo e genotype or phenotype** for the screening, evaluation, and management of patients for cardiovascular disease as a **non-covered benefit and as investigational**.

Blue Advantage does not approve or deny procedures, services, testing, or equipment for our members. Our decisions concern coverage only. The decision of whether or not to have a certain test, treatment or procedure is one made between the physician and his/her patient. Blue Advantage administers benefits based on the members' contract and medical policies. Physicians should always exercise their best medical judgment in providing the care they feel is most appropriate for their patients. Needed care should not be delayed or refused because of a coverage determination.

Key Points:

The most recent literature review was updated through November 7, 2016.

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 systematic reviews, meta-analyses and 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 Adult Treatment Panel III (ATP III) guidelines document 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 summarized the steps necessary to determine utility of a novel cardiac risk factor. Three steps were required:

- Standardization of the measurement of the risk factor.
- 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.
- 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.

Systematic Reviews

A Health Technology Assessment (HTA) performed for the U.K. National Institute for Health Research on strategies for monitoring lipid levels in patients at risk or with cardiovascular disease (CVD) was published in 2015. The HTA included a systematic review of predictive associations for CVD events. Studies were included if they had at least 12 months of follow-up and 1000 participants. Results were stratified by use of statins and primary versus secondary prevention. For populations not taking statins, 90 publications reporting 110 cohorts were included and for populations taking statins, and 25 publications reporting 28 cohorts were included. In populations not taking statins, the ratio of apolipoprotein B (apo B) to apolipoprotein AI (apo AI) was mostly strongly associated with the outcome of CVD events (hazard ratio [HR], 1.35; 95% confidence interval [CI], 1.22 to 1.5) although the hazard ratios for Apo B, total cholesterol/high-density lipoprotein (HDL), and low-density lipoprotein (LDL)/HDL all had overlapping confidence intervals with the hazard ratio for apo B/apo AI. In populations taking statins, insufficient data were available to estimate the association between apo B or apo AI with CVD events.

Thanassoulis et al in 2014 reported on a meta-analysis of seven placebo-controlled statin trials to evaluate the relationship of statin-induced reductions in lipid levels to reduction of coronary heart disease risk. Each of the trials included LDL-C, non-HDL-C, and apoB values assessed at baseline and one-year follow-up. In both frequentist and Bayesian meta-analyses, reductions in apoB were more closely related to coronary heart disease risk reduction from statins than low-density lipoprotein-cholesterol (LDL-C) or non-high-density lipoprotein-cholesterol (HDL-C).

In 2013, van Holten et al reported on a systematic review of 85 articles with 214 meta-analyses to compare serological biomarkers for risk of cardiovascular disease (CVD). Predictive potential for primary CVD events was strongest with lipids with a ranking from high to low found with: C-reactive protein, fibrinogen, cholesterol, apolipoprotein B, the apolipoprotein A/apolipoprotein

B ratio, high density lipoprotein, and vitamin D. Markers associated with ischemia were more predictive of secondary cardiovascular events and included from high to low result: cardiac troponins I and T, C-reactive protein, serum creatinine, and cystatin C. A strong predictor for stroke was fibrinogen.

Tzoulaki et al, in 2013, reported on meta-analyses on biomarkers for CVD risk to examine potential evidence of bias and inflation of result in the literature. Included in the evaluation were 56 meta-analyses with 49 reporting statistically significant results. Very large heterogeneity was seen in nine meta-analyses, and small study effects were seen in 13 meta-analyses. Significant excess of studies with statistically significant results was found in 29 meta-analyses (52%). The authors report only 13 of the meta-analyses with statistically significant results had more than 1000 cases and no evidence of large heterogeneity, small-study effects, or excess significance.

In a 2012 systematic review, Willis and colleagues evaluated whether validated cardiovascular disease risk scores can identify patients at risk for CVD for participation in more intensive intervention programs for primary prevention. Sixteen papers on five studies were included in the systematic review. Reviewers were unable to perform a meta-analysis due to the heterogeneity of the studies. The evidence was considered not strong enough to draw definitive conclusions, but the reviewers note lifestyle interventions with higher intensity may have potential for lowering CVD risk.

Apolipoprotein B

Apo B as a Predictor of Cardiovascular Risk

In 2012 Robinson et al published results of a Bayesian random-effects meta-analysis of RCTs to compare the effectiveness of lowering apo B versus low-density lipoprotein cholesterol (LDL-C) and non-high-density lipoprotein (HDL) cholesterol (HDL-C) for reducing CVD, coronary heart disease and stroke risk. Included in the analysis were 131,134 patients from 25 RCTs including 12 trials on statins, five on niacins, four on fibrates, one on simvastatin– ezetimibe, one on aggressive versus standard LDL and blood pressure targets and one on ileal bypass surgery. In the analysis of all trials, each apo B decrease of 10 mg/dL in resulted in a 6% decrease in major CVD risk and a 9% decrease in coronary heart disease risk prediction, but stroke risk was not decreased. Decreased apolipoprotein (apo) B levels were not superior to decreased non-HDL in decreasing CVD (Bayes factor [BF], 2.07) and coronary heart disease risk (BF=1.45) prediction. When non-HDL cholesterol plus LDL-C decrease were added to apo B decrease, CVD risk prediction improved slightly (BF=1.13) but not coronary heart disease risk prediction (BF=1.03) and stoke risk prediction worsened (BF=0.83). In summary, the addition of apo B decrease did not consistently add information to LDL, non-HDL or LDL/non-HDL decreases to improve CVD risk prediction when analyzed across lipid-modifying treatments of all types. Sniderman et al reported on 9345 acute myocardial infarction patients compared to 12,120 controls in the standardized case-control INTERHEART study. The authors reported discordance in the levels of cholesterol contained in apo B and non-HDL-C. In contrast to the Robinson study above, apoB was found to be more accurate than non-HDL-C as a marker for cardiovascular risk.

The Emerging Risk Factors Collaboration published a patient-level meta-analysis of 37 prospective cohort studies enrolling 154,544 individuals. Risk prediction was examined for a variety of traditional and nontraditional 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 to 1.29). 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 Quebec Cardiovascular Study 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 five 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 to 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) 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 ($p<0.001$) and 1.69 in women ($p<0.001$).

A cohort study of 15,632 participants from the Women's Health Initiative 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 to 3.72) compared to LDL-C (1.62; 95% CI, 1.17 to 2.25), after adjusting for traditional cardiovascular risk factors.

The Copenhagen City Heart Study was a prospective cohort study of 9,231 asymptomatic persons from the Danish general population followed up for eight 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 (risk ratio [RR], 1.4; 95% CI, 1.1 to 1.8 for men; RR=1.5; 95% CI, 1.1 to 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, 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 ten 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.

Kappelle et al 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 6948 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 to 1.48). This hazard ratio was not significantly different from the total cholesterol/HDL-C ratio of 1.24 (95% CI, 1.18 to 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 et al 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 two 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 two of nine 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 et al used data from 3322 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. In 2016, Pencina et al used data from 2966 participants of the Framingham Offspring cohort that were ages 40 to 75 years in the fourth examination cycle and did not have CVD, triglycerides greater than 400 mg/dL or missing data on model covariates. They calculated the differences between observed apo B and expected apo B based on linear regression models of LDL-C and non-HDL-C. These differences were added to a Cox model to predict new onset CHD adjusting for standard risk factors (age, sex, systolic blood pressure, antihypertensive treatment, smoking, diabetes, HDL-C and LDL-C or non-HDLC). The difference between observed and expected apo B was associated with future CHD events. The adjusted hazard ratio for the difference based on the apo B and LDL-C model was 1.26 (95% CI, 1.15 to 1.37) for each standard deviation increase beyond expected apo B levels. For the difference based on the apo B and non-HDL-C model, the hazard ratio was 1.20 (95% CI, 1.11 to 1.29). The discrimination C statistic for predicting new onset CHD from a model with standard risk factors was 0.72 (95% CI, 0.70 to 0.75). The C statistic improved very slightly but with overlapping confidence intervals to 0.73 (95% CI, 0.71 to 0.76) after adding the difference based on the apo B and LDL-C model to the standard risk factors and increased to 0.73 (0.71 to 0.75) after adding the difference based on the apo B and non-HDL-C model.

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.

Boekholdt et al published an individual patient-level meta-analysis of on-treatment levels of traditional and nontraditional lipids as a measure of residual risk. A total of eight studies enrolling 62,154 participants were included. The adjusted hazard ratio for each one standard deviation (SD) increase in apo B was 1.14 (95% CI, 1.11 to 1.18), which was not significantly different from LDL-C (hazard ratio [HR], 1.13; 95% CI, 1.10 to 1.17; p=0.21). The hazard ratio for HDL-C was 1.16 (95% CI, 1.12 to 1.19), which was significantly greater than LDL-C or apo B (p=0.002). In a subsequent report from this meta-analysis, Boekholdt et al evaluated the LDL-C, non-HDL-C, and apoB levels of 38,153 patients allocated to the statin therapy groups. Despite statin therapy, reductions in levels of LDL-C, non-HDL-C, and apoB from baseline to one year showed large interindividual variation.

In 2013, Ballantyne et al reported on a post hoc analysis of 682 patients with acute coronary syndrome from the randomized, Phase III study Limiting Undertreatment of Lipids in Acute Coronary Syndrome with Rosuvastatin (LUNAR) study. The LUNAR subanalysis examined apo B in relation to LDL-cholesterol and non-HDL cholesterol under intensive statin therapy with rosuvastatin or atorvastatin. The treatment target for apo B of 80mg/dL correlated with LDL-cholesterol of 90 mg/dL and non-HDL-cholesterol of 110mg/dL at baseline and with LDL-cholesterol of 74 mg/dL and non-HDL-cholesterol of 92mg/dL with statin therapy. Independent of triglyceride status, non-HDL-cholesterol was found to have a stronger correlation with apo B than LDL-cholesterol and could be an adequate surrogate of apo B during statin therapy.

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. Baseline LDL- and HDL-cholesterol, as well as levels of apo B, were predictive of future coronary events. However, in the treatment group, posttreatment levels of LDL-C and HDL-C were not predictive of subsequent risk, while posttreatment apo-B levels were predictive.

In the Long-Term Intervention with Pravastatin in Ischemic Disease (LIPID) trial, 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 to 3.64, p=0.008) was higher than that for LDL-C (1.20; 95% CI, 1.00 to 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 combined data from two 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 to 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 to 1.32), while the total/HDL-C was not. In another publication that reported on the TNT study, the on-treatment apo B level was also a significant predictor of future events (adjusted HR=1.19; 95% CI, 1.11 to 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 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.

Section Summary: Apolipoprotein B

The evidence suggests that apo B provides independent information on risk assessment for cardiovascular disease and that apo B may be 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. However, some meta-analyses have concluded that apo B is not a better predictor of cardiac risk than HDL or non-HDL combined with LDL. There is also 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.

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 antilipid 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.

It is not yet possible to conclude that the use of apo B levels will improve outcomes 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.

Apolipoprotein AI

Apo AI 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. Risk prediction was examined for a variety of traditional and nontraditional lipid markers. For apo AI, evidence from 26 studies on 139,581 individuals reported that apo AI was an independent risk factor for reduced cardiovascular risk, with an adjusted hazard ratio for cardiovascular events of 0.87 (95% CI, 0.84 to 0.90). 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 Apolipoprotein-Related Mortality Risk Study (AMORIS) followed up 175,000 Swedish men and women for 5.5 years and reported that decreased apo AI 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. This study also reported that levels of apo AI, as well as the ratio of apo B/apo AI, were strong predictors of CAD events.

The Copenhagen City Heart Study was a prospective cohort study of 9,231 asymptomatic persons from the Danish general population. The apo B/apo AI 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 AI ratio with that of traditional lipid measures, with use of the AUC for classifying cardiovascular events. The apo B/apo AI 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 et al published a prospective cohort study of 7044 elderly men enrolled in the Whitehall Cardiovascular Cohort from London, England. Measurements of apolipoprotein levels were performed on 5344 of these individuals, and patients were followed up for a mean of 6.8 years. The authors reported that the apo B/apo AI ratio was also a significant independent predictor (HR=1.54; 95% CI, 1.27 to 1.87), with similar predictive ability compared to the total cholesterol/HDL ratio (HR=1.57; 95% CI, 1.32 to 1.86).

The addition of the apo B/apo AI ratio to the Framingham risk model 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. In this analysis, individuals with apo AII 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 to 0.90).

Ridker et al compared the predictive ability of apo AI and the ratio of apo B/apo AI to standard lipid measurements. Measurements of apo AI and the apo B/apo AI 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 to 2.38) for apo AI, compared to 2.32 (95% CI,

1.64 to 3.33) for HDL-C. The hazard ratio for the ratio of apo B/apo AI was 3.01 (95% CI, 2.01 to 4.50), compared with a hazard ratio of 3.18 (95% CI, 2.12 to 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 et al 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 two 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 two of nine 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 et al used data from 3322 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 AI 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 AI ratio did not provide any incremental predictive value over traditional measures.

A nested case-control study, performed within the larger EPIC-Norfolk cohort study, evaluated the predictive ability of apo B/apo AI 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 six years and 1511 control patients without CAD. The authors reported that the apo B/apo AI ratio was an independent predictor of cardiovascular events after controlling for traditional lipid risk factors and the Framingham risk score (adjusted OR=1.85; 95% CI, 1.15 to 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).

Apo AI as a Treatment Target

A number of studies have evaluated the utility of the apo B/apo AI ratio as a marker of treatment response in RCTs of statin treatment. Kastelein et al combined data from two RCTs, the TNT and IDEAL trials, to compare the relationship between response to lipids, apo B/apo AI 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 AI was a significant predictor of events (HR=1.24; 95% CI, 1.17 to 1.32) while the total/HDL cholesterol was not.

The PROVE-IT TIMI study randomized 4162 patients with acute coronary syndrome (ACS) to standard statin therapy or intensive statin therapy. While the on-treatment ratio of apo B/apo AI ratio was a significant predictor of cardiac events (HR for each standard deviation [SD]

increment, 1.10; 95% CI, 1.01 to 1.20), it was not superior to LDL-C (HR=1.20, 95% CI, 1.07 to 1.35) or the total cholesterol/HDL ratio (HR=1.12; 95% CI, 1.01 to 1.24) as a predictor of cardiac events.

Preliminary studies of infusions of reconstituted apo A1 have demonstrated plaque regression in a small number of patients with acute coronary syndrome. Based on this research, there is interest in developing synthetic apo A1 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.

Section Summary: Apolipoprotein AI

The current evidence generally indicates that measurement of apo AI, and the apo B/apo AI 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 AI 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. 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 AI and the apo B/apo AI 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 AI to clinical decision making, both in risk assessment and treatment response, are currently not available. Apo AI 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 (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 AI mimetics are currently in development for this purpose.

Apolipoprotein E

Apo E as a Predictor of Cardiovascular Disease

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.

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. 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 TC and LDL-C levels.

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 ten years. 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.

A meta-analysis published by Bennet et al 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 to 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 to 1.13).

In 2016, Sofat et al published a meta-analysis of three studies of circulating apo E and CVD events. The method for selecting the studies was not described. The three studies included 9587 participants and 1413 CVD events. In the pooled analysis, there was no association of apo E with CVD events. The unadjusted odds ratio for CVD events for a standard deviation increase in apo E concentration was 1.02 (95% CI, 0.96 to 1.09). After adjustment for other cardiovascular risk factors, the odds ratio for CVD for a standard deviation increase in apo E concentration was 0.97 (95% CI 0.82, 1.15).

Apo E as a Predictor of Response to Therapy
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. 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.

Chiodini et al 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 3304 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.

In 2008, additional published studies were identified that evaluated apo E genetic status as a predictor of response to lipid-lowering therapy. Donnelly et al reported on 1383 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 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% to 47% of the variation in response to treatment explained by baseline LDL, compared to only 1% of the variation explained by apo E status.

Section Summary: Apolipoprotein E

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.

B-Type or Brain Natriuretic Peptide (BNP)

The use of BNP levels for monitoring and managing established heart failure patients has been frequently studied and has demonstrated value. Studies on the use of BNP for determining cardiovascular risk in the asymptomatic population, however, are limited. In the Early Identification of Subclinical Atherosclerosis by Noninvasive Imaging Research [EISNER] study, Shaw and colleagues evaluated BNP and coronary artery calcium levels in 2458 asymptomatic adults. BNP levels ranging from 40 to 99.9 and greater than or equal to100 pg/m had a 2.2 to 7.5 relative hazard for a cardiovascular event compared to BNP levels of less than 40 pg/ml (p<0.001). Other large population cohort studies have shown a relationship between elevations in BNP levels and future risks of cardiovascular events or heart failure. In a cohort study of 5067, Melander et al found adding C-reactive protein and BNP to a risk model of conventional factors increased the C-statistic for cardiovascular events by 0.007 (p=0.04) and for coronary events by 0.009 (p=0.08). In a cohort study of 3346 patients without heart failure, Wang et al found BNP levels above the 80th percentile (20.0 pg/mL for men and 23.3 pg/mL for women) were

associated with multivariable-adjusted hazard ratios of 1.62 for death (p=0.02), 1.76 for a first major cardiovascular event, (p=0.03), 1.91 for atrial fibrillation (p=0.02), 1.99 for stroke or transient ischemic attack (p=0.02), and 3.07 for heart failure (p=0.002). However, any gains over use of conventional risk factors appear to be minimal.

Section Summary: Brain Natriuretic Peptide

BNP levels appear to be associated with cardiovascular risks. However, no evidence was identified demonstrating that the use of BNP testing in clinical care improves outcomes.

Cystatin C

Ito et al evaluated the value of adding cystatin C to Framingham risk score (FRS) variables to predict cardiovascular disease risk in 6653 adults without clinical cardiovascular disease from the Multi-Ethnic Study of Atherosclerosis. Cardiovascular risk prediction did not improve with the addition of cystatin C to FRSs. Lee and colleagues conducted a meta-analysis of 14 studies consisting of 22,509 participants from predominantly high-cardiovascular-risk patients to evaluate the relationship between elevated cystatin C levels and cardiovascular disease risk. Higher levels of cystatin C were associated with greater risk of cardiovascular disease (RR=2.62; 95% CI, 2.05 to 3.37; p<0.001), coronary heart disease (RR=1.72; 95% CI, 1.27 to 2.34; p<0.001), and stroke (RR=1.83; 95% CI, 1.12 to 3.00; p=0.02) after adjustment for known cardiovascular risk factors. In 2015, Luo et el reported results of a meta-analysis of studies evaluating the role between cystatin C and cardiovascular and all-cause mortality in the general population. The study included nine prospective studies (total N=39,854 subjects). Across the six studies reporting cardiovascular mortality-specific outcomes, the pooled adjusted hazard ratio of cardiovascular mortality, comparing the highest and lowest cystatin C categories, was 2.74 (95% CI, 2.04 to 3.68; p=0.021).

Section Summary: Cystatin C

Several meta-analyses report that higher levels of cystatin C are associated with higher cardiovascular risk and higher risk of cardiovascular death. In contrast, in one large cohort, cystatin C did not improve risk prediction of cardiovascular disease. No evidence was identified demonstrating that the use of cystatin C testing in clinical care improves outcomes.

Fibrinogen

Kengne et al evaluated data from nine prospective, community-based cohorts from the British and Scottish general population-based health surveys. In the analysis of a total of 33,091 adults, of whom 1006 had diabetes, fibrinogen was found to be positively associated with a higher risk of cardiovascular disease by 34% (95% CI, 26% to 42%) and all-cause mortality by 30% (95% CI, 26% to 35%). The relationship with cardiovascular mortality and a higher fibrinogen produced hazard ratios (95% CI) of 1.48 (1.21 to 1.81) in subjects with diabetes and 1.31 (1.23 to 1.39) in those without diabetes. The interaction between fibrinogen and cardiovascular disease risk was not significantly different between the diabetic and nondiabetic populations (p=0.47). Despite any improved predictive accuracy, the addition of fibrinogen to established risk factors was reported to not be clinically important.

In 2014, Willeit et al reported results of a patient-level meta-analysis from 20 prospective studies to assess the association between a number of inflammatory markers, including fibrinogen, and atherosclerosis among patients without preexisting CVD. Included studies were prospective

cohort studies from the PROG-IMT collaboration, which included participants from the general population and reported at least two visits with measurements of common carotid artery intima-media thickness (CCA-IMT) as a marker of preclinical atherosclerosis, along with at least one inflammatory marker (hsCRP, leukocyte count, and/or fibrinogen). Overall, the authors included 20 studies with 49,087 participants, of which 13 studies (35,096 participants) reported fibrinogen levels. In cross-sectional analysis, a one SD higher baseline fibrinogen level was associated with higher CCA-IMT (mean, 0.0073 mm; 95% CI, 0.0047 to 0.0097; p<0.001). However, in longitudinal analysis, neither the baseline level of any of the inflammatory markers evaluated nor their progression was associated with progression of CCA-IMT.

Other studies have found an association between fibrinogen and cardiovascular risk including the EPIC-Norfolk cohort study and the Fibrinogen Studies Collaboration. In a report from the Fibrinogen Studies Collaboration, it was noted that fibrinogen levels increased with age and were linked to established risk factors such as triglycerides, smoking and body mass index.

Section Summary: Fibrinogen
Reports from a number of cohort studies suggest that fibrinogen levels are associated with cardiovascular risk. However, no evidence was identified demonstrating that the use of fibrinogen testing in clinical care improves outcomes.

Leptin
Sattar et al reported on a prospective study of 5661 men and a systematic review of seven prospective studies to evaluate the relationship between leptin and cardiovascular disease. Leptin levels in the top third had an odds ratio for coronary heart disease of 1.25 (95% CI, 0.96 to 1.62) compared to the bottom third. After adjusting for body mass index (BMI), this decreased to 0.98 (95% CI, 0.72 to 1.34) suggesting any association of leptin with cardiovascular disease is largely dependent on BMI.

In 2014, Zeng et al reported results of a meta-analysis of studies reporting the association between leptin levels and risk of CHD or stroke. The meta-analysis included 8 nested case-control studies with 1980 patients and 11,567 controls. In pooled analysis, leptin levels were significantly associated with pathogenic risk of CHD (OR=1.90; 95% CI, 1.06 to 3.43; p=0.032) and pathogenic risk of stroke (OR=2.14; 95% CI, 1.48 to 3.08; p<0.001).

Section Summary: Leptin
Two meta-analyses suggest that leptin levels are associated with CHD and stroke, although this association may be dependent on BMI. No evidence was identified demonstrating that the use of leptin testing in clinical care improves outcomes.

HDL Particle Size/Concentration
In the JUPITER RCT, 10,886 patients without cardiovascular disease were randomized to rosuvastatin or placebo and followed for a median of two years. Before randomization and one year after, levels of LDL-C, HDL-C, apolipoprotein A-I (apo A-I) and nuclear magnetic resonance (NMR) measured HDL size and HDL particle (HDL-P) numbers were evaluated. Statistically significant changes in the median and 25th and 75th percentile values of HDL measures between baseline and year one values occurred in the rosuvastatin and placebo groups for all levels (p<0.001) except for apo A-I and HDL-P size in the placebo group, which were not

significantly different (p=0.09 and 0.74, respectively). Changes in the rosuvastatin group were all statistically significant when compared with placebo for LDL-C, HDL-C, apo A-I, and HDL-P size and number (p<0.001 for all). In the placebo group, inverse associations with cardiovascular disease and HDL-C, apo A-1 and HDL-P were seen. HDL-P number in the rosuvastatin group had a greater association with cardiovascular disease (HR=0.73, 95% CI, 0.57 to 0.93, p=0.01) than HDL-C (HR=0.82, 95% CI, 0.63 to 1.08, p=0.16) or apo A-1 (HR=0.86, 95% CI, 0.67 to 1.10, p=0.22). This association remained after adjusting for HDL-C (HR=0.72, 95% CI, 0.53 to 0.97, p=0.03). HDL size was not significantly associated with cardiovascular disease in risk-factor-adjusted models.

Section Summary: HDL Particle Size/Concentration

One RCT has evaluated the association of HDL particle size and number as measured by NMR with residual cardiovascular disease risk. While this study found an association with HDL-P (but not HDL size) and cardiovascular disease, this does not demonstrate how NMR-measured HDL-P number would be used to change clinical management beyond information provided by traditional lipid measures. Therefore, there is no evidence that HDL size or HDL-P number measurement improves health outcomes.

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. 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 evaluated the ability of "nontraditional" lipid risk factors, including LDL size, to predict subsequent CAD events in a prospective cohort study of 2155 men followed up for five 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 six-fold increased risk of CAD, whereas when small LDL particles were not present, elevated apolipoprotein B levels were associated with only a two-fold increase in risk.

In 2005, Tzou et al examined the clinical value of "advanced lipoprotein testing" in 311 randomly selected adults participating in the Bogalusa Heart Study. 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. 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.

Superko et al 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.

These studies generally confirmed that small, dense LDL is impacted preferentially by fibrate treatment and possibly also by statin therapy. 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. 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. 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.

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. 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 1100 nmol/L, 1400 nmol/L, and 1800 nmol/L, respectively.

Mora et al 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 to 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 to 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.

Rosenson and Underberg conducted a systematic review of studies on lipid-lowering pharmacotherapies in 2013 to evaluate changes in LDL particles pre- and post-treatments. Reductions in mean LDL particles occurred in 34 of the 36 studies evaluated. Percentage reductions of LDL particles in several statin studies were smaller than reductions in LDL-C. LDL particles and apo B changes were comparable in studies. The authors suggest the differences in LDL particle reductions with different lipid-lowering therapies demonstrates potential areas of residual cardiovascular risk that can be addressed with LDL particle monitoring.

In 2014, Toth et al reported on an analysis of LDL-C and LDL-P levels and cardiovascular risk using commercial insurance and Medicare claims data on 15,569 high-risk patients from the HealthCore Integrated Research Database (HIRD). For each 100 nmol/L increase in LDL-P level, there was a 4% increase in risk of a coronary heart disease event (HR=1.04; 95% CI, 1.02 to1.05, p<0.000). A comparative analysis, using 1:1 propensity score matching of 2094 patients from the LDL-C target cohort (LDL-C level <100 mg/dL without a LDL-P level) and a LDL-P target cohort (LDL-P <1000 nmol/L and LDL-C of any level) found a lower risk of coronary heart disease or stroke in patients who received LDL-P measurement and were presumed to have received more intensive lipid-lowering therapy (HR=0.76; 95% CI, 0.61 to 0.96; at 12 months). A comparison of smaller LDL-P target groups at 24 (n=1242) and 36 (n=705) months showed similar reductions in coronary heart disease and stroke (HR=0.78, 95% CI, 0.62 to 0.97 and HR=0.75, 95% CI, 0.58 to 0.97, respectively).

Section Summary: LDL Subclass and LDL Particle Size/Concentration

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.

Lipoprotein A

Lipoprotein A as a Predictor of Cardiovascular Risk

Numerous prospective RCTs, 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. Risk prediction was examined for a variety of traditional and nontraditional 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 to 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 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 to 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 nonlipid risk factors. As part of the Framingham offspring study, lp(a) levels were measured in 2191 asymptomatic men between the ages of 20 and 54 years. 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 ten years. 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.

Several RCTs on lipid-lowering therapies have found lp(a) is associated with residual cardiovascular risk. In a subgroup analysis of 7746 white patients from the JUPITER study, median lp(a) levels did not change in either group of patients randomized to treatment with rosuvastatin or placebo during a median two-year follow-up. Lp(a) was independently associated with a residual risk of cardiovascular disease despite statin treatment (adjusted HR=1.27; 95% CI: 1.01 to 1.59, p=0.04). The LIPID RCT randomized 7863 patients to pravastatin or placebo. Patients were followed for a median six years. Lp(a) concentrations did not change significantly

at one year. Baseline lp(a) concentration was associated with total coronary heart disease events ($p<0.001$), total cardiovascular disease events ($p=0.002$), and coronary events ($p=0.03$). The AIM-HIGH study, lp(a) levels in 1440 patients at baseline and on simvastatin plus placebo or simvastatin plus extended-release niacin were significantly predictive of CV events with HRs ranging from 1.18 to 1.25.

Kamstrup et al 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 to 7.7) for women and 3.7 (95% CI, 1.7 to 8.0) in men. Tzoulaki et al reported data from the Edinburgh Artery Study, which was a population cohort study that followed up 1592 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 to 2.2) for the highest one-third versus the lowest one-third.

Zakai et al evaluated 13 potential biomarkers for independent predictive ability compared to established risk factors, using data from 4510 individuals followed up for nine years in the Cardiovascular Health Study. The lp(a) was one of seven 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 to 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. 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 2800 patients with known angina pectoris. 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, most prospective studies have indicated that lp(a) is an independent risk factor for stroke. In one prospective cohort study, Rigal et al reported that an elevated lp(a) level was an independent predictor of ischemic stroke in men (OR=3.55; 95% CI, 1.33 to 9.48) but not in women (OR=0.42; 95% CI, 0.12 to 1.26). In the ARIC prospective cohort study of 14,221 participants, elevated lp(a) was a significant independent predictor of stroke in African-American women (RR=1.84; 95% CI, 1.05 to 3.07) and white women (RR=2.42; 95% CI, 1.30 to 4.53) but not in African-American men (RR=1.72; 95% CI, 0.86 to 3.48) or white men (RR=1.18; 95% CI, 0.47 to 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 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 to 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 to 1.53; $p=0.18$).

Several meta-analyses have also examined the relationship between lp(a) levels and cardiovascular risk. Bennet et al synthesized the results of 31 prospective studies with at least one 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 to 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. 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 to 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 to 3.63).

A patient-level meta-analysis of 36 prospective studies published between 1970 and 2009 included 126,634 participants. 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 to 1.18) for coronary heart disease and 1.10 (95% CI, 1.02 to 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, there were three separate loci identified for increased lp(a) levels. Genetic variants were identified at two of these loci that were independently associated with coronary disease (OR=1.70; 95% CI, 1.49 to 1.95, and OR=1.92; 95% CI, 1.48 to 2.49). This finding strongly implies that elevated lp(a) levels are causative of coronary disease, as opposed to simply being associated.

Lipoprotein A as Treatment Target

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.

Section Summary: Lipoprotein A

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.

Summary of Evidence
For individuals who are asymptomatic with risk of CVD who receive novel cardiac biomarker testing (e.g., apo B, apo AI, lipoprotein [a], apo E, subclasses of LDL and HDL, B-type natriuretic peptide, cystatin C, fibrinogen, leptin), the evidence includes systematic reviews, meta-analyses, and large, prospective cohort studies. Relevant outcomes ARE overall survival, other test performance measures, change in disease status, morbid events, and medication use. The evidence from cohort studies and meta-analyses of these studies has suggested that some of these markers are associated with increased cardiovascular risk and may provide incremental accuracy in risk prediction. In particular, apo B and apo AI have been identified as adding some incremental predictive value. However, it has not been established whether 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. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals with hyperlipidemia managed with lipid-lowering therapy who receive testing with novel cardiac biomarker testing (e.g., apo B, apo AI, lipoprotein [a], apo E, subclasses of LDL and HDL, B-type natriuretic peptide, cystatin C, fibrinogen, and leptin), the evidence includes analyses of the intervention arm(s) of lipid-lowering medication trials. Relevant outcomes include overall survival, change in disease status, morbid events, and medication use. In particular, apo B, apo AI, and apo E have been evaluated as markers of success of lipid-lowering treatment success, and evidence from the intervention arms from several randomized controlled trials has suggested that these markers are associated with treatment success. However, there is no direct evidence that using markers other than LDL and HDL as a lipid-lowering treatment target leads to improved health outcomes. The evidence is insufficient to determine the effects of the technology on health outcomes.

Practice Guidelines and Position Statements
American College of Cardiology and American Heart Association
ACC/AHA published guidelines in 2013 for the assessment of cardiovascular risk. Pooled cohort equations for estimating arteriosclerotic cardiovascular disease (ASCVD) were developed from sex- and race-specific proportional hazards models that included covariates of age, treated or untreated systolic blood pressure level, total cholesterol and high-density lipoprotein (HDL) cholesterol levels, current smoking status, and history of diabetes. Additional risk factors evaluated included diastolic blood pressure, family history of ASCVD, moderate or severe chronic kidney disease, and body mass index. None of these variables significantly improved discrimination for 10-year hard ASCVD risk prediction. Further research using state of the art statistical techniques (including net reclassification improvement and integrative discrimination index) are needed to examine the utility of novel biomarkers when added to these new pooled cohort equations in different populations and patient subgroups. The document states that future updates might include guidance on whether on-treatment markers such as apo B, Lp(a), or low-density lipoprotein (LDL) particles are useful for guiding treatment decisions.

European Society of Cardiology et al

The 2012 guidelines on cardiovascular disease prevention from the European Society of Cardiology (ESC) and Other Societies on Cardiovascular Disease Prevention in Clinical Practice indicate apo B can be a substitute for LDL-cholesterol but it doesn't improve risk assessment and isn't readily available. The use of lipoprotein (a) isn't justified as a treatment target or for screening the general population.

ESC 2016 guidelines for cardiovascular risk prevention in clinical practice have recommendations for lipid control based on LDL-C levels and targets. The guidelines indicated that 'there is no evidence that apo B is a better predictor of CVD [cardiovascular disease] than LDL-C." It also stated that while it is beyond doubt that the apo B/apo AI ratio is one of the strongest predictors of CVD, there is insufficient evidence to supports its use as a treatment goal.

National Heart, Lung, and Blood Institute

The National Heart, Lung, and Blood Institute's (NHLBI's) 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. 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. There was a 2004 update to these guidelines which discussed the result of clinical trials of statin therapy.

In 2013, NHLBI published a systematic evidence review from the Cholesterol Expert Panel on managing blood cholesterol in adults. The review was used to develop joint guidelines by the American College of Cardiology and American Heart Association (ACC/AHA) on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults.

American Diabetes Association and American College of Cardiology Foundation

In 2008, a publication from a consensus conference of the American Diabetes Association and the American College of Cardiology Foundation addressed lipoprotein management in patients with cardiometabolic risk. These guidelines included specific recommendations for incorporating apo B testing into clinical care for high-risk patients and 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, even if target LDL has been achieved.

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.

National Lipid Association

The National Lipid Association (NLA) recommendations for patient-centered management of dyslipidemia were published in 2014. These recommendations stated that non-HDL-C and LDL-C should be the primary targets for therapy and that apo B is an optional, secondary target for therapy. NLA favored non-HDL-C over apo B because it is universally available and because

apo B has not consistently shown superiority in predicting atherosclerotic cardiovascular disease risk.

Canadian Cardiovascular Society
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. 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. These guidelines were updated in 2006.

National Institute for Health and Care Excellence
The National Institute for Health and Care Excellence (NICE) issued guidance on risk assessment and reduction, including lipid modification, of cardiovascular disease in 2014 and most recently updated in 2016. The guidelines recommend measuring a full lipid profile including total cholesterol, HDL, non-HDL, and triglycerides before starting lipid lowering therapy for primary prevention of CVD. The guidelines also recommend measurement of total cholesterol, HDL, non-HDL, and triglycerides for primary and secondary prevention in people who have been started on high intensity statins at 3 months of treatment aiming for 40% reduction in non-HDL. Apo B and other nontraditional risk factors were not discussed as part of risk assessment or treatment targets.

U.S. Preventive Services Task Force Recommendations
The USPSTF issued recommendations in 2009 (USPSTF 2009) 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".

Key Words:
Apolipoprotein A-1, apo A-I, apolipoprotein B, apo B. apolipoprotein E, apo E, B-type natriuretic peptide, cardiac risk factors, novel cardiovascular risk assessment, cystatin C, fibrinogen, HDL subclass testing, HDL subclassification, high density lipoprotein subclassification, LDL subclass, leptin, lipoprotein A., lipoprotein, small-density, management of cardiovascular disease, small-density lipoproteins, small-diameter lipoproteins, lp(a)

Approved by Governing Bodies:
Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Lipid and non–lipid biomarker tests are available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

Benefit Application:

Coverage is subject to member's specific benefits. Group specific policy will supersede this policy when applicable.

Current Coding:

CPT Codes:

81401	Molecular pathology procedure, Level 2 (e.g., 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat) includes—*APOE (apolipoprotein E)* (e.g., hyperlipoproteinemia type III, cardiovascular disease, Alzheimer disease), common variants (e.g., *2, *3, *4)
82172	Apolipoprotein, each
82397	Chemiluminescent assay
82610	Cystatin C
82664	Electrophoretic technique, not elsewhere specified
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise 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 (e.g., electrophoresis, ultracentrifugation)
83704	Lipoprotein, blood; quantitation of lipoprotein particle number(s) (e.g., by nuclear magnetic resonance spectroscopy), includes lipoprotein particle subclass(es), when performed
83880	Natriuretic peptide
84181	Protein; Western Blot, with interpretation and report, blood or other body fluid
84999	Unlisted chemistry procedure
85384	Fibrinogen; activity
85385	Fibrinogen; antigen
0423T	Secretory type II phospholipase A2 (sPLA2-IIA) **(Effective 01/01/2016)**

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This policy is intended to be used for adjudication of claims (including pre-admission certification, pre-determinations, and pre-procedure review) in Blue Cross and Blue Shield's administration of plan contracts.