2.04.65	Biomarker Testing in Risk Assessment and Management of Cardiovascular Disease		
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Section:	2.0 Medicine	**Page:**	Page 1 of 56

Policy Statement

I. Measurement of **any** of the following nontraditional lipid and non-lipid biomarkers as an adjunct to low-density lipoprotein cholesterol in the risk assessment and management of cardiovascular disease is considered **investigational.**
   A. Apolipoprotein AI
   B. Apolipoprotein B
   C. Apolipoprotein E
   D. B-type natriuretic peptide
   E. Cystatin C
   F. Fibrinogen
   G. High-density lipoprotein (HDL) subclass
   H. Leptin
   I. Lipoprotein (a)
   J. Low-density lipoprotein (LDL) subclass

II. Measurement of lipoprotein-associated phospholipase A_2 is considered **investigational**.

III. Cardiovascular disease risk panels, consisting of multiple individual biomarkers intended to assess cardiac risk (other than simple lipid panels, see Policy Guidelines section), are considered **investigational**.

NOTE: Refer to Appendix A to see the policy statement changes (if any) from the previous version.

Policy Guidelines

A simple lipid panel is generally composed of the following lipid measures:

- Total cholesterol
- Low-density lipoprotein cholesterol
- High-density lipoprotein cholesterol
- Triglycerides

Certain calculated ratios (e.g., total/high-density lipoprotein cholesterol) may also be reported as part of a simple lipid panel.

Other types of lipid testing (i.e., apolipoproteins, lipid particle number or particle size, lipoprotein [a]) are not considered components of a simple lipid profile.

This policy does not address the use of panels of biomarkers in the diagnosis of acute myocardial infarction.

Genetic Counseling
Experts recommend formal genetic counseling for individuals who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some individuals; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic

testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Coding

Apolipoprotein B

There is no specific CPT code for measurement of apolipoprotein (apo) B. The following CPT code might be used:

- **82172**: Apolipoprotein, each

Apo E Phenotyping or Genotyping

There is no specific code for apo E phenotyping or genotyping. The following CPT code may be used for phenotyping:

- **84181**: Protein; Western Blot, with interpretation and report, blood or other body fluid

High-Density Lipoprotein Subclass

There is no CPT code for subclassification specific to high-density lipoprotein (HDL). The following CPT code may be used:

- **82664**: Electrophoretic technique, not elsewhere specified
- **83701**: Lipoprotein, blood; high resolution fractionation and quantitation of lipoproteins including lipoprotein subclasses when performed (e.g., electrophoresis, ultracentrifugation)

Lipoprotein Particle Number and Subclass Quantitation

The following CPT code is for lipoprotein particle number and subclass quantification by nuclear magnetic resonance spectroscopy that is also not specific to HDL:

- **83704**: Lipoprotein, blood; quantitation of lipoprotein particle number(s) (e.g., by nuclear magnetic resonance spectroscopy), includes lipoprotein particle subclass(es), when performed

Quantitation of Lipoprotein Levels

The following CPT codes for quantitation of lipoprotein levels are available:

- **0052U**: Lipoprotein, blood, high resolution fractionation and quantitation of lipoproteins, including all five major lipoprotein classes and subclasses of HDL, LDL, and VLDL by vertical auto profile ultracentrifugation
- **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

Lipoprotein (a)

There is a specific CPT code for lipoprotein (a) testing:

- **83695:** Lipoprotein (a)

B-type Natriuretic Peptide

There is a specific CPT code for B-type natriuretic peptide testing:

- **83880**: Natriuretic peptide

Cystatin C

Testing for cystatin C is reported with the following CPT code:

- **82610:** Cystatin C

Fibrinogen

There are 2 CPT codes for fibrinogen testing:

- **85384:** Fibrinogen; activity
- **85385:** Fibrinogen; antigen

Leptin

There is no specific CPT code for leptin testing. According to laboratory websites, the following CPT codes might be used:

- **82397**: Chemiluminescent assay

The following Category 1 code is a direct single step method, for the quantification of small dense low-density lipoprotein cholesterol:

- **83722**: Lipoprotein, direct measurement; small dense LDL cholesterol

Description

Numerous lipid and non-lipid biomarkers have been proposed as potential risk markers for cardiovascular disease (CVD). Biomarkers assessed herein include apolipoprotein B, apolipoprotein AI, apolipoprotein E, B-type natriuretic peptide, cystatin C, fibrinogen, high-density lipoprotein subclass, leptin, low-density lipoprotein subclass, lipoprotein(a), and lipoprotein-associated phospholipase A_2 (Lp-PLA_2). These biomarkers have been studied as alternatives or additions to standard lipid panels for risk stratification in CVD or as treatment targets for lipid-lowering therapy. Cardiovascular risk panels refer to different combinations of cardiac markers that are intended to evaluate the risk of CVD. There are numerous commercially available risk panels that include different combinations of lipids, noncardiac biomarkers, measures of inflammation, metabolic parameters, and/or genetic markers. Risk panels report the results of multiple individual tests, as distinguished from quantitative risk scores that combine the results of multiple markers into a single score.

Related Policies

- Genetic Testing for Alzheimer Disease

Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

Regulatory Status

Multiple assay methods for cardiac risk marker components, such as lipid panels and other biochemical assays, have been cleared for marketing by the U.S. Food and Drug Administration (FDA) through the 510(k) process.

In December 2014, the PLAC® Test (diaDexus), a quantitative enzyme assay, was cleared for marketing by the U.S. Food and Drug Administration (FDA) through the 510(k) process for Lp-PLA_2 activity. It was considered substantially equivalent to a previous version of the PLAC® Test (diaDexus), which was cleared for marketing by the FDA in July 2003. FDA product code: NOE.

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Components of testing panels, lipid, and non-lipid biomarker tests are available under the auspices of the CLIA. Laboratories that offer laboratory-developed tests must be licensed by the CLIA for high-complexity testing. To date, the FDA has chosen not to require any regulatory review of these tests.

Rationale

Background

Cardiovascular Disease

Cardiovascular disease (CVD) remains the single largest cause of morbidity and mortality in the developed world. Mortality from CVD has accounted for 1 in 4 deaths in the United States, and there are numerous socio-economic factors that affect CVD mortality rates.[1,] Lower-income, race, age, and behavioral factors all have a significant impact on health outcome disparities associated with CVD. As a result, accurate prediction of CVD risk is a component of medical care that has the potential to focus on and direct preventive and diagnostic activities. Current methods of risk prediction in use in general clinical care are not highly accurate and, as a result, there is a potential unmet need for improved risk prediction instruments.

Risk Assessment

Although treatment for elevated coronary disease risk with statins targets cholesterol levels, selection for treatment involves estimation of future coronary artery disease (CAD) risk using well-validated prediction models that use additional variables.

Components of CVD risk include family history, cigarette smoking, hypertension, and lifestyle factors such as diet and exercise. Also, numerous laboratory tests have been associated with CVD risk, most prominently lipids such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL). These clinical and lipid factors are often combined into simple risk prediction instruments, such as the Framingham Risk Score.[2,] The Framingham Risk Score provides an estimate of the 10-year risk for developing cardiac disease and is currently used in clinical care to determine the aggressiveness of risk factor intervention, such as the decision to treat hyperlipidemia with statins.

Many additional biomarkers, genetic factors, and radiologic measures have been associated with an increased risk of CVD. Over 100 emerging risk factors have been proposed as useful for refining estimates of CVD risk.[3,4,5,] Some general categories of these potential risk factors are as follows:

- **Lipid markers.** In addition to LDL and HDL, other lipid markers may have predictive ability, including the apolipoproteins, lipoprotein (a) (Lp[a]), lipid subfractions, and/or other measures.
- **Inflammatory markers.** Many measures of inflammation have been linked to the likelihood of CVD. High-sensitivity C-reactive protein (hs-CRP) is an example of an inflammatory marker; others include fibrinogen, interleukins, and tumor necrosis factor.
- **Metabolic syndrome biomarkers.** Measures associated with metabolic syndromes, such as specific dyslipidemic profiles or serum insulin levels, have been associated with an increased risk of CVD.
- **Genetic markers.** A number of variants associated with increased thrombosis risk, such as the 5,10-methylene tetrahydrofolate reductase (*MTHFR*) variant or the prothrombin gene

variants, have been associated with increased CVD risk. Also, numerous single nucleotide variants have been associated with CVD in large genome-wide studies.

Risk Panel Testing

CVD risk panels may contain measures from 1 or all of the previous categories and may include other measures not previously listed such as radiologic markers (carotid medial thickness, coronary artery calcium score). Some CVD risk panels are relatively limited, including a few markers in addition to standard lipids. Others include a wide variety of potential risk factors from a number of different categories, often including both genetic and nongenetic risk factors. Other panels are composed entirely of genetic markers.

Some examples of commercially available CVD risk panels are as follows:

- **CV Health Plus Genomics™ Panel (Genova Diagnostics):** apolipoprotein (apo) E; prothrombin; factor V Leiden; fibrinogen; HDL; HDL size; HDL particle number; homocysteine; LDL; LDL size; LDL particle number; Lp(a); lipoprotein-associated phospholipase A2 (Lp-PLA2); *MTHFR* gene; triglycerides; very-low-density lipoprotein (VLDL); VLDL size; vitamin D; hs-CRP.
- **CV Health Plus™ Panel (Genova Diagnostics):** fibrinogen; HDL; HDL size; HDL particle number; homocysteine; LDL; LDL size; LDL particle number; lipid panel; Lp(a); Lp-PLA2; triglycerides; VLDL; VLDL size; vitamin D; hs-CRP.
- **CVD Inflammatory Profile (Cleveland HeartLab):** hs-CRP, urinary microalbumin, myeloperoxidase, Lp-PLA2, F_2 isoprostanes.
- **Applied Genetics Cardiac Panel:** genetic variants associated with CAD: cytochrome p450 variants associated with the metabolism of clopidogrel, ticagrelor, warfarin, beta-blockers, rivaroxaban, prasugrel (2C19, 2C9/*VKORC1*, 2D6, 3A4/3A5), factor V Leiden, prothrombin gene, *MTHFR* gene, *APOE* gene.
- **Genetiks Genetic Diagnosis and Research Center Cardiovascular Risk Panel:** factor V Leiden, factor V R2, prothrombin gene, factor XIII, fibrinogen-455, plasminogen activator inhibitor-1, platelet glycoprotein (GP) IIIA variant human platelet antigen (HPA)-1 (PLA1/2), *MTHFR* gene, angiotensin-converting enzyme insertion/deletion, apo B, apo E.

In addition to panels that are specifically focused on CVD risk, a number of commercially available panels include markers associated with cardiovascular health, along with a range of other markers that have been associated with inflammation, thyroid disorders and other hormonal deficiencies, and other disorders. An example of these panels is:

- **Advanced Health Panel (Thorne):** total cholesterol, HDL, LDL, triglycerides, HDL ratios, non-HDL cholesterol, LDL particle number, small LDL, medium LDL, LDL pattern, LDL peak size, large HDL, apo A1, apo B, Lp(a), cortisol, hs-CRP, homocysteine, glucose, hemoglobin A1c, insulin, homeostatic model assessment for insulin resistance, free T4, free T3, thyroid-stimulating hormone, reverse T3, dehydroepiandrosterone sulfate, estradiol, follicle stimulating hormone, luteinizing hormone, sex hormone binding globulin, total testosterone, free testosterone, albumin, globulin, albumin/globulin ratio, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, total bilirubin, total serum protein, blood urea nitrogen, creatinine, blood urea nitrogen/creatinine ratio, estimated glomerular filtration rate form creatinine, estimated glomerular filtration rate from cystatin C, cystatin C, fibrinogen, platelet count, white cell count, absolute neutrophils, lymphocytes, absolute lymphocytes, monocytes, absolute monocytes, eosinophils, absolute eosinophils, basophils, absolute basophils, red blood cell count, hemoglobin, hematocrit, mean platelet volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, red cell distribution width, folate, vitamin B12, vitamin D, red blood cell magnesium, calcium, carbon dioxide, chloride, potassium, sodium, ferritin, iron total iron binding capacity, omega-3 index, omega-6 to omega-3 ratio,

arachidonic acid, eicosapentaenoic acid, eicosapentaenoic acid/arachidonic acid ratio, docosahexaenoic acid, free fatty acids.[6,]

Low-density Lipoproteins and Cardiovascular Disease

Low-density lipoproteins (LDLs) have been identified as the major atherogenic lipoproteins and have long been identified by the National Cholesterol Education Project as the primary target of cholesterol-lowering therapy. An LDL particle consists 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 CAD occur in subjects with "normal" levels of total cholesterol and LDL-C. Thus, there is considerable potential to improve the accuracy of current cardiovascular risk prediction models.

Other non-lipid markers have been identified as being associated with CVD, including B-type natriuretic peptide, cystatin C, fibrinogen, and leptin. These biomarkers may have a predictive role in identifying CVD risk or in targeting therapy. In the United States, social, biological, and environmental disparities exist in the prevalence, morbidity, and mortality rates that are associated with CVD.[7,] Population subgroups that are most significantly adversely affected by such disparities include Black and Hispanic Americans, individuals with low socioeconomic status, and individuals who live in rural settings.

Lipid Markers

Apolipoprotein B

Apolipoprotein (Apo) B is the major protein moiety of all lipoproteins, except for HDL. The most abundant form of apo B, large B or B_{100}, constitutes the apo B found in LDL and very-low density LDL. Because LDL and very-low density LDL each contain 1 molecule of apo B, the measurement of apo B reflects the total number of these atherogenic particles, 90% of which are LDL. Because LDL particles can vary in size and in cholesterol content, for a given concentration of LDL-C, there can be a wide variety in 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 LDL concentration.

Apolipoprotein AI

HDL contains 2 associated apolipoproteins (i.e., apo AI, apo AII). HDL particles can also be classified by whether they contain apo AI only or they contain apo AI and apo AII. All lipoproteins contain apo AI, and some also contain apo AII. Because 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 the evaluation of cardioprotective, or "good," cholesterol. In addition, the ratio of 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 is the primary apolipoprotein found in very-low density LDLs and chylomicrons. Apolipoprotein E is the primary binding protein for LDL receptors in the liver and is thought to play an important role in lipid metabolism. The apolipoprotein E (*APOE*) gene is polymorphic, consisting of 3 epsilon alleles (e2, e3, e4) that code for 3 protein isoforms, known as E2, E3, and E4, which differ from one another by one amino acid. These molecules mediate lipid metabolism through their different interactions with LDL receptors. The genotype of apo E alleles can be assessed by gene amplification techniques, or the *APOE* phenotype can be assessed by measuring plasma levels of apo E.
It has been proposed that various *APOE* genotypes are more atherogenic than others and that *APOE* measurement may provide information on the risk of CAD beyond traditional risk factor

measurement. It has also been proposed that the *APOE* 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. The *APOE* genotype may be a factor that determines an individual's degree of response to interventions such as statin therapy.

High-Density Lipoprotein 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 or density and/or on apolipoprotein composition. Using size or 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.

An alternative to measuring the concentration of subclasses of HDL (e.g., HDL_2, HDL_3) is a direct measurement of HDL particle size and/or number. Particle size can be measured by nuclear magnetic resonance (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 AI has used HDL particle number as a surrogate, based on the premise that each HDL particle contains a single apo AI molecule.

Low-Density Lipoprotein Subclass

Two main subclass patterns of LDL, called A and B, have been described. In subclass pattern A, particles have a diameter larger than 25 nm and are less dense, while in subclass pattern B, particles have a diameter less than 25 nm and a higher density. Subclass pattern B is a common 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 apo B, and low levels of HDL. This lipid profile is commonly seen in type 2 diabetes and is a 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) to also include high normal blood pressure, insulin resistance, increased levels of inflammatory markers such as C-reactive protein, and a prothrombotic state. The presence of the metabolic syndrome is considered by Adult Treatment Panel 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 the risk for CAD independent of the total LDL level. Also, some drugs may cause a greater shift in lipid profiles 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 whether to use a combination of 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 for LDL-C is not a direct measure of LDL, but, chosen for its convenience, measures the amount of cholesterol incorporated into LDL particles. Because 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 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 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 than larger particles. Therefore,

for patients with elevated numbers of LDL particles, the cardiac risk may be further enhanced when the particles are smaller versus larger.

Lipoprotein (a)

Lp (a) is a lipid-rich particle similar to LDL. The major apolipoprotein associated with LDL is Apo B; in Lp(a), however, there is an additional apo A covalently linked to apo B. The apo A molecule is structurally similar to plasminogen, suggesting that Lp(a) may contribute to the thrombotic and atherogenic basis of CVD. Levels of Lp(a) are relatively stable in individuals over time but vary up to 1000-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 Lp(a) levels. Therefore, it has been proposed that levels of Lp(a) may be an independent risk factor for CAD.

Non-Lipid Markers

B-type or Brain Natriuretic Peptide

Brain natriuretic peptide (BNP, also called B-type natriuretic peptide) is an amino acid polypeptide secreted primarily by the ventricles of the heart when the 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. Brain natriuretic peptide 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 secreted from all functional cells in 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 CVD and all-cause mortality.

Leptin

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

Lipoprotein-associated Phospholipase A_2

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), also known as platelet-activating factor acetylhydrolase, is an enzyme that hydrolyzes phospholipids and is primarily associated with LDLs. Accumulating evidence has suggested that Lp-PLA_2 is a biomarker of CAD and may have a proinflammatory role in the progression of atherosclerosis. Recognition that atherosclerosis represents, in part, an inflammatory process has created considerable interest in the measurement of pro-inflammatory factors as part of cardiovascular disease risk assessment.

Interest in Lp-PLA_2 as a possible causal risk factor for CAD has generated the development and testing of Lp-PLA_2 inhibitors as a new class of drugs to reduce the risk of CAD. However, clinical trials of Lp-PLA_2 inhibitors have not shown significant reductions in CAD endpoints.[8,9,10,] Furthermore, assessment of Lp-PLA_2 levels has not been used in the selection or management of subjects in the clinical trials.

Literature Review

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance

of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Promotion of greater diversity and inclusion in clinical research of historically marginalized groups (e.g., People of Color [African-American, Asian, Black, Latino and Native American]; LGBTQIA (Lesbian, Gay, Bisexual, Transgender, Queer, Intersex, Asexual); Women; and People with Disabilities [Physical and Invisible]) allows policy populations to be more reflective of and findings more applicable to our diverse members. While we also strive to use inclusive language related to these groups in our policies, use of gender-specific nouns (e.g., women, men, sisters, etc.) will continue when reflective of language used in publications describing study populations.

Nontraditional Biomarkers

A large body of literature has accumulated on the utility of nontraditional lipid risk factors in the prediction of future cardiac events. The evidence reviewed herein consists of systematic reviews, meta-analyses, and large, prospective cohort studies that have evaluated the association between these lipid markers and cardiovascular outcomes. A smaller amount of literature is available on the utility of these markers as a marker of treatment response. Data on treatment responses are 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 noted that, to determine their clinical significance, emerging risk factors should be evaluated against the following criteria:[11,]

- 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

It is preferable, but not necessary, that modification of the risk factor in clinical trials will have shown a reduction in risk.

Representative Systematic Reviews

A 2015 health technology assessment conducted for the National Institute for Health Research assessed strategies for monitoring lipid levels in patients at risk or with cardiovascular disease (CVD).[12,] The assessment 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 the 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, 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 most strongly associated with the outcome of CVD events (hazard ratio [HR], 1.35; 95% confidence interval [CI], 1.22 to 1.5) although the HRs for apo B, total cholesterol (TC)/high-density lipoprotein (HDL), and low-density lipoprotein (LDL)/HDL all had overlapping CIs with the HR for apo B/apo AI. In populations taking statins, insufficient data were available to estimate the association between apo B or apo AI and CVD events.

Thanassoulis et al (2014) reported on a meta-analysis of 7 placebo-controlled statin trials evaluating the relation between statin-induced reductions in lipid levels and reduction of coronary heart disease (CHD) risk.[13,] Each trial included LDL cholesterol (LDL-C), non-HDL cholesterol (HDL-C), and apo B

values assessed at baseline and 1-year follow-up. In both frequentist and Bayesian meta-analyses, reductions in apo B were more closely related to CHD risk reduction from statins than LDL-C or non-HDL-C.

Van Holten et al (2013) reported on a systematic review of 85 articles with 214 meta-analyses to compare serologic biomarkers for risk of CVD.[14,] Predictive potential for primary CVD events was strongest with lipids, with a ranking from high to low found with: C-reactive protein (CRP), fibrinogen, cholesterol, apo B, the apo A/apo B ratio, HDL, 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, CRP, serum creatinine, and cystatin C. A strong predictor for stroke was fibrinogen.

Tzoulaki et al (2013) reported on meta-analyses of biomarkers for CVD risk to examine potential evidence of bias and inflation of results in the literature.[15,] Included in the evaluation were 56 meta-analyses, with 49 reporting statistically significant results. Very large heterogeneity was seen in 9 meta-analyses, and small study effects were seen in 13 meta-analyses. Significant excess of studies with statistically significant results was found in 29 (52%) meta-analyses. Reviewers reported only 13 meta-analyses with statistically significant results that had more than 1000 cases and no evidence of large heterogeneity, small-study effects, or excess significance.

In a systematic review, Willis et al (2012) evaluated whether validated CVD risk scores could identify patients at risk for CVD for participation in more intensive intervention programs for primary prevention.[16,] Sixteen articles reporting on 5 studies were selected. Reviewers were unable to perform a meta-analysis due to the heterogeneity of studies. The evidence was not considered strong enough to draw definitive conclusions, but reviewers noted that lifestyle interventions with higher intensity might have the potential for lowering CVD risk.

Asymptomatic Individuals with Risk of Cardiovascular Disease

Clinical Context and Test Purpose

The purpose of nontraditional cardiac biomarker testing in individuals who are asymptomatic with risk of CVD is to inform a decision whether nontraditional cardiac biomarker testing improves CVD diagnosis and treatment decisions.

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals who are asymptomatic with risk of CVD.

Interventions

The intervention being considered is nontraditional cardiac biomarker testing.

Comparators

Comparators of interest include routine care without biomarker testing.

Outcomes

The general outcomes of interest are overall survival (OS), other test performance measures, change in disease status, morbid events, and medication use.

Study Selection Criteria

Methodologically credible studies were selected using the following principles:

- The study population represents the population of interest. Eligibility and selection are described.
- The test is compared with a credible reference standard.

- If the test is intended to replace or be an adjunct to an existing test; it should also be compared with that test.
- Studies should report sensitivity, specificity, and predictive values. Studies that completely report true- and false-positive results are ideal. Studies reporting other measures (eg, receiver operating characteristic, area under operating characteristic, C statistic, likelihood ratios) may be included but are less informative.
- Studies should also report reclassification of the diagnostic or risk category.

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Apolipoprotein B

Systematic Review

Robinson et al (2012) published results of a Bayesian random-effects meta-analysis of RCTs to compare the effectiveness of lowering apo B versus LDL-C and non-HDL-C for reducing CVD, CHD, and stroke risk.[17,] Selected for analysis were 131,134 patients from 25 RCTs including 12 trials on statins, 5 on niacin, 4 on fibrates, 1 on simvastatin plus ezetimibe, 1 on aggressive versus standard LDL and blood pressure targets, and 1 on ileal bypass surgery. In the analysis of all trials, each apo B decrease of 10 mg/dL resulted in a 6% decrease in major CVD risk and a 9% decrease in CHD risk prediction, but stroke risk was not decreased. Decreased apo B levels were not superior to decreased non-HDL levels in decreasing CVD (Bayes factor [BF], 2.07) and CHD risk (BF, 1.45) prediction. When non-HDL-C plus LDL-C decrease were added to apo B decrease, CVD risk prediction improved slightly (BF, 1.13) but not CHD risk prediction (BF, 1.03) and stroke risk prediction worsened (BF, 0.83). In summary, any 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.
The Emerging Risk Factors Collaboration (2012) published a patient-level meta-analysis of 37 prospective cohort studies enrolling 154,544 patients.[18,] Risk prediction was examined for a variety of traditional and nontraditional lipid markers. For apo B, evidence from 26 studies (n=139,581) reported that apo B was an independent risk factor for cardiovascular events (Table 1). On reclassification analysis, when apo B and apo AI were substituted for traditional lipids, there was no improvement in risk prediction. In fact, there was a slight worsening in the predictive ability, as evidenced by a -0.0028 decrease in the C statistic (p<.001), and a -1.08% decrease in the net reclassification improvement (p<.01).

Observational Studies

The Quebec Cardiovascular Study (1996) 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 2155 men followed for 5 years.[19,] Elevated levels of apo B were found to be an independent risk factor for ischemic heart disease after adjustment for other lipid parameters (Table 1; study 2). 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 was another prospective cohort study (2001) that followed 175,000 Swedish men and women presenting for routine outpatient care over a mean of 5.5 years.[20,] This study found that apo B was an independent predictor of CAD events and was superior to LDL-C levels in predicting risk, not only for the entire cohort but also for all subgroups examined. Relative risks (RR) for the highest quartile of apo B levels were 1.76 in men (p<.001) and 1.69 in women (p<.001).

A cohort study (2005) of 15,632 participants from the Women's Health Initiative provided similar information in women.[21,] In this analysis, the HR for developing CHD in the highest versus the lowest quintiles was greater for apo B (2.50; 95% CI, 1.68 to 3.72) than LDL-C (1.62; 95% CI, 1.17 to 2.25), after adjusting for traditional cardiovascular risk factors.

The Copenhagen City Heart Study (2007) prospectively evaluated a cohort of 9231 asymptomatic persons from the Danish general population followed for 8 years.[22,] Subjects with total apo B levels in the top one-third (top tertile) had a significantly increased RR of cardiovascular events than patients in the lowest one-third, after controlling for LDL-C and other traditional cardiovascular risk factors. 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 (0.58) than LDL-C (0.57); however, this difference in AUC was not statistically significant.

Kappelle et al (2011) used data from the prospective Prevention of Renal and Vascular End-stage Disease trial (PREVEND) cohort to evaluate the predictive value of the apo B/apo AI ratio independent of other traditional risk factors, including albuminuria and CRP.[23,] Among 6948 subjects without previous heart disease and who were not on lipid-lowering drugs, the adjusted HR (aHR) for a high apo B/apo AI ratio did not differ significantly from the TC/HDL-C ratio of 1.24 (95% CI, 1.18 to 1.29), and did not change significantly after further adjustment for triglycerides.

Pencina et al (2015) used data from 2966 participants of the Framingham Offspring Study cohort who were 40 to 75 years of age in the fourth examination cycle and did not have CVD, triglyceride levels greater than 400 mg/dL, or missing data on model covariates.[24,] They calculated the differences between observed apo B and expected apo B based on linear regression models of LDL-C and non-HDL-C levels. 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-HDL-C). The difference between observed and expected apo B was associated with future CHD events. The aHR 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 (SD) increase beyond expected apo B levels. For the difference based on the apo B and non-HDL-C model, the HR 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 CIs 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 (95% CI, 0.71 to 0.75) after adding the difference based on the apo B and non-HDL-C model.

Table 1 summarizes the results of the above apolipoprotein B studies.

Table 1. Results of Diagnostic Apolipoprotein B Studies

Study	Study Type	N	Efficacy of Apo B in Determining CVD Risk	
			HR (95% CI)	RR (95% CI)
ERFC (2012)[18,]	MA of prospective cohorts	154,544	1.24 (1.19 to 1.29)	-
Lamarche et al (1996)[19,]	Prospective cohort	2155	-	1.40 (1.2 to 1.7)
Walldius et al (2001)[20,]	Prospective cohort	175,000	-	Men: 1.76 (p<.001) Women: 1.69 (p<.001)
Ridker et al (2005)[21,]	Prospective cohort	15,632	2.50 (1.68 to 3.72)	-
Benn et al (2007)[22,]	Prospective cohort	9231	-	Men: 1.4 (1.1 to 1.8) Women: 1.5 (1.1 to 2.1)
Kappelle et al (2011)[23,]	Prospective cohort	6948	1.37 (1.26 to 1.48)	-

Study	Study Type	N	Efficacy of Apo B in Determining CVD Risk
Pencina et al (2015)[24,]	Prospective cohort	2966	1.26 (1.15 to - 1.37)

Apo B: apolipoprotein B; CI: confidence interval; CVD: cardiovascular disease; ERFC: Emerging Risk Factors Collaboration; HR: hazard ratio; MA: meta-analysis; RR: relative risk

The Atherosclerosis Risk in Communities (ARIC) study (2001), concluded that apo B did not add additional predictive information above standard lipid measures.[25,] The ARIC study followed 12,000 middle-aged adults free of CAD at baseline for 10 years. While apo B was a strong univariate predictor of risk, it did not add independent predictive value above traditional lipid measures in multivariate models.

The ratio of apo B/apo AI has also been proposed as a superior measure of the ratio of proatherogenic (i.e., "bad") cholesterol to anti-atherogenic (i.e., "good") cholesterol. This ratio may be a more accurate measure of this concept, compared with the more common TC/HDL ratio. A number of epidemiologic studies have reported that the apo B/apo AI ratio is superior to other ratios, such as TC/HDL-C and non-HDL-C/HDL-C.[26,27,] Other representative studies of the apo B/apo AI ratio are discussed next.

Some studies have tested the use of apo B in a multivariate risk prediction model with both traditional risk factors and apolipoprotein measures included as potential predictors. Ridker et al (2007) published the Reynolds Risk Score, based on data from 24,558 initially healthy women enrolled in the Women's Health Study and followed for a median of 10.2 years.[28,] Thirty-five potential predictors of CVD were considered as potential predictors, and 2 final prediction models were derived. The first was the best-fitting model statistically and included both apo B and the apo B/apo AI ratio as 2 of 9 final predictors. The second called the "clinically simplified model" substituted LDL-C for apo B and TC/HDL-C for apo B/apo AI. The authors developed this simplified model "for the purpose of clinical application and efficiency" and justified replacing the apo B and apo B/apo AI measures as a result of their high correlation with traditional lipid measures (r=0.87 and 0.80, respectively). The predictor has not been evaluated in clinical care.

Ingelsson et al (2007) used data from 3322 subjects in the Framingham Offspring Study to compare prediction models using traditional lipid measures with models using apolipoprotein and other nontraditional lipid measures.[29,] This study reported that the apo B/apo AI ratio had a similar predictive ability as 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.

Sniderman et al (2012) reported on 9345 acute myocardial infarction (MI) patients who were compared with 12,120 controls in the standardized case-control INTERHEART study.[30,] The authors reported discordance in the levels of cholesterol contained in apo B and non-HDL-C. Unlike the Robinson et al (2012) study, apo B was found to be more accurate than non-HDL-C as a marker for cardiovascular risk.

Subsection Summary: Apolipoprotein B

The evidence has suggested that apo B provides independent information on risk assessment for CVD 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 than 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 about 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 present, apo B is not

included in the models most commonly used in routine clinical care, such as the Framingham risk model.

Apolipoprotein AI

Systematic Review

In the Emerging Risk Factors Collaboration meta-analysis (2012) described above, apo AI was also examined as an independent risk factor.[18,] For apo AI, evidence from 26 studies (n=139,581 subjects) reported that apo AI was an independent risk factor for reduced cardiovascular risk (Table 2). However, as with apo B, when apo AI was substituted for traditional lipids, there was no improvement in risk prediction. In fact, there was a slight worsening in the predictive ability, evidenced by a -0.0028 decrease in the C statistic (p<.001) and a -1.08% decrease in the net reclassification improvement (p<.01).

Observational Studies

Clarke et al (2007) published a prospective cohort study of 7044 elderly men enrolled in the Whitehall Cardiovascular Cohort from England.[31,] Measurements of apolipoprotein levels were performed on 5344 of these men, and they were followed for a mean of 6.8 years. The authors reported that the apo B/apo AI ratio was a significant independent predictor (Table 2) with similar predictive ability as the TC/HDL ratio (HR, 1.57; 95% CI, 1.32 to 1.86).

Ridker et al (2007), described above, compared the predictive ability of apo AI and the apo B/apo AI ratio with standard lipid measurements.[28,] Both ratios had similar predictive ability to standard lipid measurements but were no better. The HR for future cardiovascular events was 1.75 (95% CI, 1.30 to 2.38) for apo AI compared with 2.32 (95% CI, 1.64 to 3.33) for HDL-C (Table 2). The HR for the apo B/apo AI ratio was 3.01 (95% CI, 2.01 to 4.50) compared with 3.18 (95% CI, 2.12 to 4.75) for the LDL-C/HDL-C ratio.

A nested case-control study (2007), performed within the larger European Prospective Investigation into Cancer and Nutrition-Norfolk cohort study, evaluated the predictive ability of the apo B/apo AI ratio in relation to traditional lipid measures in 25,663 patients.[32,] The case-control subgroup study enrolled 869 patients who had developed CAD during a mean follow-up of 6 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 (Table 2). However, the authors also reported that this ratio was no better than the TC/HDL ratio in discriminating between cases (AUC, 0.673) and controls (AUC, 0.670; p=.38).

Table 2. Results of Diagnostic Apolipoprotein AI Studies

Study	Study Type	N	Efficacy of Apolipoprotein AI in Determining CVD Risk	
			HR (95% CI)	OR (95% CI)
ERFC (2012)[18,]	Review of prospective cohorts	139,581	0.87 (0.84 to 0.90)	-
Clarke et al (2007)[31,]	Prospective cohort	7044	1.54 (1.27 to 1.87)	-
Ridker et al (2007)[28,]	Prospective cohort	2966	2.32 (1.64 to 3.33)	-
van der Steeg et al (2007)[32,]	Case-control	25,663	-	1.85 (1.15 to 2.98)

CI: confidence interval; CVD: cardiovascular disease; ERFC: Emerging Risk Factors Collaboration; HR: hazard ratio; OR: odds ratio.

The Apolipoprotein Mortality Risk Study (2001) followed 175,000 Swedish men and women for 5.5 years and reported that decreased apo AI was an independent predictor of CAD events.[20,] The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS [2000]) investigated lipid parameters among 6605 men and women with average LDL-C and low HDL-C levels who were

randomized to lovastatin or placebo.[33,] This study reported that apo AI levels and the apo B/apo AI ratio were strong predictors of CAD events.

The Copenhagen City Heart Study (2007) was a prospective cohort study of 9231 asymptomatic persons from the Danish general population.[22,] The apo B/apo AI ratio was reported as an independent predictor of cardiovascular events, with an HR similar to that for TC/HDL-C. This study also compared the discriminatory ability of the apo B/apo AI ratio with that of traditional lipid measures, using the AUC for classifying cardiovascular events. The apo B/apo AI ratio had a slightly higher AUC (0.59) than the TC/HDL-C ratio (0.58), but this difference was not statistically significant.

Section Summary: Apolipoprotein AI

The current evidence has generally indicated that the measurement of apo AI and the apo B/apo AI ratio are as good as or better than currently used lipid measures such as LDL and HDL. Some experts have argued 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.[33,34,] 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.

Apolipoprotein E

A large body of research has established a correlation between lipid levels and the underlying *APOE* 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.[35,36,]

Systematic Reviews

A meta-analysis published by Bennet et al (2007) summarized the evidence from 147 studies on the association between *APOE* genotypes using lipid levels and cardiac risk.[37,] Eighty-two studies with a total of 86,067 participants included data on the association between apo E and lipid levels and 121 studies reported on the association with clinical outcomes. The authors estimated that patients with the apo e2 allele had LDL levels that were approximately 31% lower than those in patients with the apo e4 allele. Compared with patients with the apo e3 allele, patients with apo e2 had an approximately 20% lower risk for coronary events (odds ratio [OR], 0.80; 95% CI, 0.70 to 0.90). Patients with the apo e4 had an estimated 6% higher risk of coronary events, which was of marginal statistical significance (OR, 1.06; 95% CI, 0.99 to 1.13).

Sofat et al (2016) published a meta-analysis of 3 studies of circulating apo E and CVD events.[38,] The method for selecting the studies was not described. The 3 studies included 9587 participants and 1413 CVD events. In a pooled analysis, there was no association between apo E and CVD events. The unadjusted OR for CVD events for each SD increase in apo E concentration was 1.02 (95% CI, 0.96 to 1.09). After adjustment for other cardiovascular risk factors, the OR for CVD for each SD increase in apo E concentration was 0.97 (95% CI, 0.82 to 1.15).

Observational Studies

Numerous studies have focused on the relation 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.[39,40,41,42,43,44,] 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.[45,46,47,48,]

Some larger observational studies have correlated *APOE* genotype with clinical disease. The ARIC study (2001) followed 12,000 middle-aged subjects free of CAD at baseline for 10 years.[25,] This study

reported that the apo e3/2 genotype was associated with carotid artery atherosclerosis after controlling for other atherosclerotic risk factors. Volcik et al (2006), also analyzing ARIC study data, reported that *APOE* polymorphisms were associated with LDL levels and carotid intima-media thickness but were not predictive of incident CAD.[49,]

Subsection Summary: Apolipoprotein E

The evidence has suggested that *APOE* 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 compared with other established and emerging clinical variables, and does not explain a large percentage of the interindividual variation in TC and LDL levels. Moreover, apo E has not been incorporated into standardized cardiac risk assessment models and was not identified as an important "emerging risk factor" in the most recent ATP III recommendations.

High-Density Lipoprotein Particle Size and Concentration

Systematic Review

Singh et al (2020) reported the results for a pooled analysis examining the association between HDL particle concentration and stroke and MI in patients without baseline atherosclerotic disease.[50,] The analysis included 15,784 patients from 4 prospective cohort studies, which included the ARIC study. A significant inverse association was reported between HDL particle concentration and stroke and MI, when comparing patients with HDL particle concentration in the fourth quartile and the first quartile (HR, 0.64; 95% CI, 0.52 to 0.78). When comparing quartile 4 with quartile 1 with regard to the individual components of the primary endpoint, a significant reduction in both MI (HR, 0.63; 95%, 0.49 to 0.81) and stroke (HR, 0.66; 95% CI, 0.48 to 0.93) was reported. There was significant heterogeneity between studies with regard to patient ethnicity and geographic location. Sub-analysis by race revealed that the significant inverse association between HDL particle concentration and stroke and MI was not seen in black populations. When comparing quartile 4 with quartile 1 among black patients, HDL particle concentration did not have an inverse association with MI (HR, 1.22; 95% CI, 0.76 to 1.98). However, the heterogeneity and uneven distribution of patients may have contributed to subgroup analyses being underpowered and the possibility of type 2 error.

Randomized Controlled Trial

In the Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER RCT) (2013), 10,886 patients without CVD were randomized to rosuvastatin or placebo and followed for a median of 2 years.[51,] Before randomization and 1 year after, levels of LDL-C, HDL-C, apo AI, and nuclear magnetic resonance (NMR)-measured HDL size and HDL particle numbers were evaluated. Statistically significant changes in the median and 25th and 75th percentile values of HDL levels between baseline and year 1 values occurred in the rosuvastatin and placebo groups for all levels ($p<.001$), except for apo AI and HDL particle size in the placebo group, which did not differ significantly ($p=.09$ and.74, respectively). Changes in the rosuvastatin group were also statistically significant compared with placebo for LDL-C, HDL-C, apo AI, and HDL particle size and number (all $p<.001$). In the placebo group, inverse associations with CVD and HDL-C, apo AI, and HDL particles were reported. HDL particle number in the rosuvastatin group had a greater association with CVD (HR, 0.73; 95% CI, 0.57 to 0.93; $p=.01$) than HDL-C (HR, 0.82; 95% CI, 0.63 to 1.08; $p=.16$) or apo AI (HR, 0.86; 95% CI, 0.67 to 1.10; $p=.22$). This association remained after adjusting for HDL-C (HR, 0.72; 95% CI, 0.53 to 0.97; $p=.03$). Size of HDL was not significantly associated with CVD in risk factor-adjusted models.

Subsection Summary: High-Density Lipoprotein Particle Size and Concentration

One RCT and a pooled analysis have evaluated the association of HDL particle size and number as measured by NMR. While these studies found an association with HDL particle concentration (but not HDL size) and CVD, it is uncertain how NMR-measured HDL particle number would be used to change clinical management beyond the information provided by traditional lipid measures. It is also

unclear whether the association between HDL particle concentration and cardiovascular events is seen in all patient populations.

Low-Density Lipoprotein Subclass and Low-Density Lipoprotein Particle Size and Concentration Observational Studies

A nested case-control study (1996) from the Physician's Health Study, a prospective cohort study of approximately 15,000 men, investigated whether LDL particle size is an independent predictor of CAD risk, particularly compared to triglyceride levels.[52,] The authors concluded that while LDL particle diameter was associated with the risk of MI, this association was not present after adjustment for triglyceride level. Only the triglyceride level was independently significant.

The Quebec Cardiovascular Study evaluated the ability of "nontraditional" lipid risk factors, including LDL size, to predict subsequent CAD events in a prospective cohort of 2155 men followed for 5 years.[19,53,] The presence of small LDL particles was associated with a 2.5-fold 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 apo B levels. In the presence of small LDL particles, elevated apo B levels were associated with a 6-fold increased risk of CAD, whereas when small LDL particles were not present, elevated apo B levels were associated with only a 2-fold increase in risk.

Tzou et al (2005) examined the clinical value of "advanced lipoprotein testing" in 311 randomly selected adults participating in the Bogalusa Heart Study.[54,] Advanced lipoprotein testing consisted of subclass patterns of LDL (i.e., 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.

Low-Density Lipoprotein Particle Size and Concentration Measured by Nuclear Magnetic Resonance

Similar to small dense lipoprotein particles, several epidemiologic studies have shown that the lipoprotein particle size and concentration measured by NMR are also associated with cardiac risk. For example, data derived from the Women's Health Study, Cardiovascular Health Study, and Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-1) trial have suggested that the number of LDL particles is an independent predictor of cardiac risk.[55,56,57,] Translating these findings into clinical practice requires setting target values for lipoprotein numbers. Proposed target values have been derived from the same data set (i.e., Framingham study) 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 numbers correspond to the same percentile values, and LDL particle concentrations corresponding to the 20th, 50th, and 80th percentile are 1100, 1400, and 1800 nmol/L, respectively.[58,]

Systematic Review

Rosenson and Underberg (2013) conducted a systematic review of studies on lipid-lowering pharmacotherapies to evaluate changes in LDL particles pre- and post-treatment.[59,] 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. Reviewers suggested the differences in LDL particle reductions with different lipid-lowering therapies demonstrated potential areas of residual cardiovascular risk that could be addressed with LDL particle monitoring.

Observational Studies

Mora et al (2009) evaluated the predictive ability of LDL particle size and number measured by NMR in participants of the Women's Health Study, a prospective cohort trial of 27,673 women followed over an 11-year period.[60,] After controlling for non-lipid factors, LDL particle number was a significant predictor of incident CVD, with an HR of 2.51 (95% CI, 1.91 to 3.30) for the highest compared with the lowest quintile. LDL particle size was similarly predictive of cardiovascular risk, with an HR of 0.64 (95% CI, 0.52 to 0.79). Compared with standard lipid measures and apolipoproteins, LDL particle size and number showed similar predictive ability but were not superior in predicting cardiovascular events.

Toth et al (2014) analyzed LDL-C and LDL particle levels and cardiovascular risk using commercial insurance and Medicare claims data on 15,569 high-risk patients from the HealthCore Integrated Research Database.[61,] For each 100 nmol/L increase in LDL particle level, there was a 4% increase in the risk of a CHD event (HR, 1.04; 95% CI, 1.02 to 1.05; p<.0001). 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 particle level) and a LDL particle target cohort (LDL particle <1000 nmol/L and LDL-C of any level) found a lower risk of CHD or stroke in patients who received LDL-C 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 particle target groups at 24 (n=1242) and 36 (n=705) months showed similar reductions in CHD (HR, 0.78; 95% CI, 0.62 to 0.97) and stroke (HR, 0.75; 95% CI, 0.58 to 0.97).

Subsection Summary: Low-Density Lipoprotein Subclass and Low-Density Lipoprotein Particle Size and Concentration

Small LDL size is a component of an atherogenic lipid profile; other components include increased triglycerides, increased apo B, and decreased HDL. Some studies have reported that LDL size is an independent risk factor for CAD, while others have reported that a shift in LDL size may be a useful marker of treatment response.

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 predictive ability beyond that provided by traditional lipid measures. Measures by NMR 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)

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 relevant literature. Table 3 summarizes the results of diagnostic Lp(a) studies that assess the HR or OR of the efficacy of Lp(a) in determining CVD risk.

Systematic Review

The Emerging Risk Factors Collaboration (2012) published a patient-level meta-analysis assessing 37 prospective cohort studies enrolling 154,544 individuals.[18,] Risk prediction was examined for a variety of traditional and nontraditional lipid markers. For Lp(a), evidence from 24 studies on 133,502 subjects reported that Lp(a) was an independent risk factor for reduced cardiovascular risk (Table 3). The addition of Lp(a) to traditional risk factors resulted in a small improvement in risk prediction, with a 0.002 increase in the C statistic. A reclassification analysis found no significant improvement in the net reclassification index (0.05%; 95% CI, -0.59% to 0.70%).

Several meta-analyses have also examined the relation between Lp(a) levels and cardiovascular risk. Bennet et al (2008) synthesized the results of 31 prospective studies with at least 1 year of follow-up and that reported data on cardiovascular death and nonfatal MI.[62,] The combined results revealed a

significant positive relationship between Lp(a) and cardiovascular risk (Table 3). This analysis reported a moderately high degree of heterogeneity in selected studies (I^2=43%), reflecting the fact that not all reported a significant positive association.

Smolders et al (2007) summarized evidence from observational studies on the relation between Lp(a) and stroke.[63,] Five prospective cohort studies and 23 case-control studies were included in this meta-analysis. Results from prospective cohort studies showed that Lp(a) level added only incremental predictive information (combined RR for the highest one-third of Lp[a], 1.22; 95% CI, 1.04 to 1.43). Results from case-control studies showed an elevated Lp(a) level was associated with an increased risk of stroke (Table 3).

Randomized Controlled Trials

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 (2014), median Lp(a) levels did not change in either group of patients randomized to treatment with rosuvastatin or placebo during a median 2-year follow-up.[64,] Lp(a) was independently associated with a residual risk of CVD despite statin treatment (Table 3). In the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglyceride and Impact on Global Health Outcomes study (2013), Lp(a) levels in 1440 patients at baseline and on simvastatin plus placebo or simvastatin plus extended-release niacin were significantly predictive of cardiovascular events (Table 3).[65,]

Observational Studies

Kamstrup et al (2008) analyzed data from the Copenhagen City Heart Study, which followed 9330 subjects from the Copenhagen general population over 10 years.[66,] This study reported on a graded increase in the risk of cardiac events with increasing Lp(a) levels. At extreme levels of Lp(a) above the 95th percentile, the aHR for MI was slightly higher for women than for men (Table 3). Tzoulaki et al (2007) reported on data from the Edinburgh Artery Study, a population cohort study that followed 1592 subjects for a mean of 17 years.[67,] They reported that Lp(a) was an independent predictor of MI (Table 3).

Zakai et al (2007) evaluated 13 potential biomarkers for independent predictive ability compared with established risk factors, using data from 4510 subjects followed for 9 years in the Cardiovascular Health Study.[68,] Lipoprotein (a) was 1 of 7 biomarkers that had incremental predictive ability above the established risk factors (Table 3).

Waldeyer et al (2017) analyzed data of 56,084 participants from Biomarkers for Cardiovascular Risk Assessment in Europe project, which followed 7 prospective population-based cohorts across Europe, with a maximum follow-up of 24 years, to characterize the association of Lp(a) concentration with major coronary events, incident CVD, and total mortality.[69,] The highest event rate of major coronary events and CVD was observed for Lp(a) levels at the 90th percentile or higher (p<.001 for major coronary events and CVD). Adjusting for age, sex, and cardiovascular risk factors, compared with Lp(a) levels in the lowest third in the 67th to 89th percentile, there were significant associations between Lp(a) levels and major coronary events (HR, 1.3; 95% CI, 1.15 to 1.46) and CVD (HR, 1.25; 95% CI, 1.12 to 1.39) (Table 3). For Lp(a) levels at the 90th percentile or higher, the aHR for the association between Lp(a) and major coronary events was 1.49 (95% CI, 1.29 to 1.73) and for the association between Lp(a) and CVD, it was 1.44 (95% CI, 1.25 to 1.65) compared with Lp(a) levels in the lowest third. There was no significant association between Lp(a) levels and total mortality.

Lee et al (2017) investigated whether elevated circulating Lp(a) level was a key determinant in predicting the incidence of major adverse cardiovascular events (MACE) among the participants of the Dallas Health Study, a multiethnic prospective cohort with a median follow-up of 9.5 years (N =3419 patients).[70,] Quartiles 4 of Lp(a) and oxidized phospholipid on apo B-100 were associated with HRs for time to MACE of 2.35 (95% CI, 1.50 to 3.69) and 1.89 (95% CI, 1.26 to 2.84), respectively, adjusting for age, sex, body mass index (BMI), diabetes, smoking, LDL, HDL-C, and triglycerides

(Table 3). The addition of major apolipoprotein(a) isoform and 3 *LPA* single nucleotide variants prevalent among White, Black, and Hispanic subjects in the model attenuated the risk, but significance was maintained for both Lp(a) and oxidized phospholipid on apo B-100.

Some researchers have hypothesized that there is a stronger relation between Lp(a) and stroke than CHD. Similar to the situation with cardiac disease, most prospective studies have indicated that Lp(a) level is an independent risk factor for stroke. In a prospective cohort study, Rigal et al (2007) reported that an elevated Lp(a) level was an independent predictor of ischemic stroke in men (Table 3).[71,]

There also may be a link between Lp(a) level as a cardiovascular risk factor and hormone status in women. Suk Danik et al (2008) reported on the risk of a first cardiovascular event over a 10-year period in 27,736 women enrolled in the Women's Health Study.[72,] After controlling for standard cardiovascular risk factors, Lp(a) levels were an independent predictor of risk in women not taking hormone replacement therapy (Table 3). However, for women who were taking hormone 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=.18).

Table 3. Results of Diagnostic Lipoprotein(a) Studies

Study	Study Type	N	Efficacy of Lp(a) in Determining CVD Risk	
			HR (95% CI)	OR (95% CI)
ERFC (2012)[18,]	SR/MA	154,544	1.13 (1.09 to 1.18)	-
Khera et al (2014)[64,]	RCT	7746	1.27 (1.01 to 1.59) p=.04	-
Albers et al (2013)[65,]	RCT	1440	1.18 to 1.25	-
Kamstrup et al (2008)[66,]	Post hoc analysis	9330	Men: 3.6 (1.7 to 7.7) Women: 3.7 (1.7 to 8.0)	-
Tzoulaki et al (2007)[67,]	Prospective cohort	1592	1.49 (1.0 to 2.2)	-
Zakai et al (2007)[68,]	Prospective cohort	4510	1.07 (1.0 to 1.12)	-
Waldeyer et al (2017)[69,]	Post hoc analysis	56,084	1.3 (1.15 to 1.46)	-
Lee et al (2017)[70,]	Prospective cohort	3419	2.35 (1.50 to 3.69)	-
Rigal et al (2007)[71,]	Prospective cohort	100	-	Men: 3.55 (1.33 to 9.48) Women: 0.42 (0.12 to 1.26)
Suk Danik et al (2008)[72,]	Prospective cohort	27,736	1.77 (1.36 to 2.30) p<.001	-
Bennet et al (2008)[62,]	SR/MA	2047	-	1.45 (1.32 to 1.58)
Smolders et al (2007)[63,]	SR/MA of Observational	56,010	-	2.39 (1.57 to 3.63)

CI: confidence interval; CVD: cardiovascular disease; ERFC: Emerging Risk Factors Collaboration; HR: hazard ratio; MA: meta-analysis; Lp(a): lipoprotein(a); OR: odds ratio; RCT: randomized control trial; SR: systematic review.

Additional Studies

Beyond the studies describing the HR or OR for the efficacy of Lp(a) and CVD summarized in Table 3, additional key studies have examined the relation between Lp(a) and CVD risk, which are summarized below.

Additional Systematic Reviews

A systematic review by Genser et al (2011) included 67 prospective studies (N=181,683) that evaluated the risk of CVD associated with Lp(a).[73,] Pooled analysis was performed on 37 studies that reported the endpoints of cardiovascular events. When grouped by design and populations, the RRs 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 CVD than with patients without the previous disease. There were no significant associations found between Lp(a) levels, overall mortality, or stroke.

A patient-level meta-analysis (2009) of 36 prospective studies published between 1970 and 2009 included 126,634 participants.[74,] Overall, the independent association between Lp(a) level and vascular disease was consistent across studies but modest in size. The combined RR, adjusted for age, sex, and traditional lipid risk factor, was 1.13 (95% CI, 1.09 to 1.18) for CHD and 1.10 (95% CI, 1.02 to 1.18) for ischemic stroke. There was no association between Lp(a) levels and mortality.

Additional Randomized Controlled Trials

The Lipid Research Clinics Coronary Primary Prevention Trial (1994), 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 controlling for other lipid and non-lipid risk factors.[75,]
The LIPID RCT (2013) randomized 7863 patients to pravastatin or placebo.[76,] Patients were followed for a median of 6 years. Lipoprotein (a) concentrations did not change significantly at 1 year. Baseline Lp(a) concentration was associated with total CHD events (p<.001), total CVD events (p=.002), and coronary events (p=.03).

Additional Observational Studies

As part of the Framingham Offspring Study, Lp(a) levels were measured in 2191 asymptomatic men between the ages of 20 and 54 years.[77,] After a mean follow-up of 15 years, there were 129 CHD 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 CHD (ie, before age 55 years). The ARIC study (2001) evaluated the predictive ability of Lp(a) in 12,000 middle-aged subjects free of CAD at baseline who were followed for 10 years.[25,] Lipoprotein (a) levels were significantly higher among patients who developed CAD than among those who did not, and Lp(a) levels were an independent predictor of CAD above traditional lipid measures.

In the ARIC prospective cohort study of 14,221 participants, elevated Lp(a) was a significant independent predictor of stroke in Black 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 Black men (RR, 1.72; 95% CI, 0.86 to 3.48) or White men (RR, 1.18; 95% CI, 0.47 to 2.90).[78,]

Fogacci et al (2017) examined whether serum Lp(a) levels could predict long-term survival in 1215 adults with no CVD at enrollment and similar general cardiovascular risk profiles from Brisighella Heart Study cohort in Italy.[79,] Subjects were stratified into low (n=865), intermediate (n=275), and high (n=75) cardiovascular risk groups using an Italian-specific risk chart. Subjects at high and intermediate cardiovascular risk aged 56 to 69 years (regardless of sex) and women aged 40 to 55 years with a low cardiovascular risk profile who had lower Lp(a) levels showed statistically significant lower cardiovascular mortality (p<.05) and longer survival time (p<.05) during the 25-year follow-up. The authors constructed a receiver operating characteristic curve for each cardiovascular risk group using Lp(a) as a test variable and death as a state variable and identified serum Lp(a) as an independent long-term cardiovascular mortality prognostic indicator for subjects at high cardiovascular risk (AUC, 0.63; 95% CI, 0.50 to 0.76; p=.049) and for women at intermediate cardiovascular risk (AUC, 0.7; 95% CI, 0.52 to 0.79; p=.034).

Some studies, however, have failed to demonstrate such predictive ability. In the Physicians' Health Study (1993), 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.[80,] Authors found that the distribution of Lp(a) levels between the groups was identical. The European Concerted Action on Thrombosis and Disabilities study (2000), a trial of secondary prevention, evaluated Lp(a) as a risk factor for coronary events in 2800 patients with known angina pectoris.[81,] In this study, Lp(a) levels did not differ significantly among patients who did and did not have subsequent events, suggesting that Lp(a) levels were not useful risk markers in this population.

Genetic studies have examined the association between various genetic loci and Lp(a) levels, and Mendelian randomization studies have examined whether Lp(a) level is likely to be causative for CAD. In a 2009 study, 3 separate loci were identified for increased Lp(a) levels.[82,] Genetic variants identified at 2 of these loci were independently associated with coronary disease (OR, 1.70; 95% CI, 1.49 to 1.95; 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.

Subsection Summary: Lipoprotein (a)

A large amount of epidemiologic evidence has determined that Lp(a) is an independent risk factor for CVD. 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.

B-Type or Brain Natriuretic Peptide

Observational Studies

The use of B-type or brain natriuretic peptide (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 study, Shaw et al (2009) evaluated BNP and coronary artery calcium levels in 2458 asymptomatic adults.[83,] Levels of BNP ranging from 40 to 99.9 pg/mL and from 100 pg/mL or higher had a 2.2 to 7.5 relative hazard for a cardiovascular event compared with BNP levels less than 40 pg/mL (p<.001). Other large population cohort studies have shown a relationship between elevations in BNP levels and future risks of cardiovascular events or heart failure. Wu et al (2022) assessed the value of cardiac troponins and amino terminal B type cardiac natriuretic peptide (NT-proBNP) in 2 different cohorts of asymptomatic patients (n=4102; n=2538).[84,] Study investigators found that cardiac marker data correctly reclassified risk upwards in 6.7% of patients and downwards in 3.3% of patients; the overall C statistic for discrimination of the primary endpoint (composite of all first cardiovascular events) increased from 0.755 to 0.771 (+0.016 ; p=.01). In a cohort study (N=5067), Melander et al (2009) found adding CRP and BNP to a risk model of conventional factors increased the C statistic for cardiovascular events by 0.007 (p=.04) and for coronary events by 0.009 (p=.08).[85,] In a cohort study of 3346 patients without heart failure, Wang et al (2004) found that BNP levels above the 80th percentile (20.0 pg/mL for men, 23.3 pg/mL for women) were associated with multivariable aHRs of 1.62 for death (p=.02), 1.76 for a first major coronary event, (p=.03), 1.91 for atrial fibrillation (p=.02), 1.99 for stroke or transient ischemic attack (p=.02), and 3.07 for heart failure (p=.002).[86,] However, any gains over the use of conventional risk factors appear to be minimal.

Subsection Summary: B-Type or Brain Natriuretic Peptide

Levels of BNP 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 (2011) evaluated the value of adding cystatin C to Framingham Risk Score variables to predict CVD risk in 6653 adults without CVD from the Multi-Ethnic Study of Atherosclerosis.[87,] Cardiovascular risk prediction did not improve with the addition of cystatin C to Framingham Risk Score variables.

Lee et al (2010) conducted a meta-analysis of 14 studies (N=22,509) with predominantly high cardiovascular risk patients to evaluate the relation between elevated cystatin C levels and CVD risk.[88,] Higher levels of cystatin C were associated with greater risk of CVD (RR, 2.62; 95% CI, 2.05 to 3.37; p<.001), CHD (RR, 1.72; 95% CI, 1.27 to 2.34; p<.001), and stroke (RR, 1.83; 95% CI, 1.12 to 3.00; p=.02) after adjusting for known cardiovascular risk factors. Luo et al (2015) reported on a meta-analysis of studies evaluating the relation between cystatin C and cardiovascular and all-cause mortality in the general population.[89,] Reviewers included 9 prospective studies (N=39,854 subjects). Across the 6 studies reporting cardiovascular mortality-specific outcomes, the pooled aHR of cardiovascular mortality, comparing the highest and lowest cystatin C categories, was 2.74 (95% CI, 2.04 to 3.68; p=.021).

Subsection Summary: Cystatin C

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

Fibrinogen

Systematic Reviews

Kengne et al (2013) evaluated data from 9 prospective, community-based cohorts from the British and Scottish general population-based health surveys.[90,] In the analysis of 33,091 adults, 1006 of whom had diabetes, fibrinogen was positively associated with a higher risk of CVD by 34% (95% CI, 26% to 42%) and all-cause mortality by 30% (95% CI, 26% to 35%). The relation between cardiovascular mortality and higher fibrinogen produced HRs of 1.48 (95% CI, 1.21 to 1.81) in subjects with diabetes and 1.31 (95% CI, 1.23 to 1.39) in those without diabetes. The interaction between fibrinogen levels and CVD risk did not differ significantly between the diabetic and nondiabetic populations (p=.47). Despite improved predictive accuracy, the addition of fibrinogen to established risk factors was not reported to be clinically important.

Willeit et al (2016) reported on 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.[91,] Selected were prospective cohort studies from the PROG-IMT collaboration, which included participants from the general population and reported at least 2 visits with measurements of common carotid artery intima-media thickness as a marker of preclinical atherosclerosis, along with at least 1 inflammatory marker (high-sensitivity-CRP, leukocyte count, and/or fibrinogen). Overall, reviewers included 20 studies (N=49,087 participants), of which 13 studies (n=35,096) reported fibrinogen levels. In a cross-sectional analysis, a 1 SD higher baseline fibrinogen level was associated with common carotid artery intima-media thickness (mean, 0.0073 mm; 95% CI, 0.0047 to 0.0097 mm; p<.001). However, in longitudinal analysis, neither the baseline level of any of the inflammatory markers evaluated nor their progression was associated with the progression of common carotid artery intima-media thickness.

Observational Studies

Other studies have found an association between fibrinogen and cardiovascular risk, including the European Prospective Investigation into Cancer and Nutrition-Norfolk cohort study[92,] and the Fibrinogen Studies Collaboration.[93,94,] In a 2007 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 BMI.[94,]

Subsection Summary: Fibrinogen

Reports from a number of cohort studies and subsequent systematic review/meta-analysis, have suggested 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

Systematic Reviews

Sattar et al (2009) reported on a prospective study of 5661 men and a systematic review of 7 prospective studies to evaluate the relationship between leptin and CVD.[95,] Leptin levels in the top third had an odds for CHD of 1.25 (95% CI, 0.96 to 1.62) compared with the bottom third. After adjusting for BMI, the odds decreased to 0.98 (95% CI, 0.72 to 1.34), suggesting an association of leptin with CVD is largely dependent on BMI.

Zeng et al (2014) conducted a meta-analysis of studies reporting on the association between leptin levels and risk of CHD or stroke.[96,] The meta-analysis included 8 nested case-control studies with 1980 patients and 11,567 controls. In a pooled analysis, leptin levels were significantly associated with pathogenic risk of CHD (OR, 1.90; 95% CI, 1.06 to 3.43; p=.032) and pathogenic risk of stroke (OR, 2.14; 95% CI, 1.48 to 3.08; p<.001).

Yang et al (2017) conducted a systematic review of case-control and cohort studies that assessed leptin concentration and CHD risk.[97,] Thirteen epidemiologic studies totaling 4257 CVD patients and 26,710 controls were included. Adjusting for cardiovascular risk factors, there was no statistically significant association between leptin concentration and CHD risk (OR, 1.16; 95% CI, 0.97 to 1.40). The association did not change when analyses were restricted to high-quality studies (OR, 1.07; 95% CI, 0.96 to 1.19) for CHD. In a subgroup meta-analysis, a high leptin level was not independently associated with CHD in either female (OR, 1.03; 95% CI, 0.86 to 1.23) or male patients (OR, 1.09; 95% CI, 0.95 to 1.26).

Subsection Summary: Leptin

Two meta-analyses have suggested that leptin levels are associated with CHD and stroke, although this association may depend on BMI. Another meta-analysis suggested no significant association between leptin concentration and CHD risk. No evidence was identified demonstrating that the use of leptin testing in clinical care improves outcomes.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Section Summary: Asymptomatic Individuals with Risk of Cardiovascular Disease

The evidence for asymptomatic individuals with risk of CVD who receive nontraditional cardiac biomarker testing includes systematic reviews, meta-analyses, and large, prospective cohort studies. 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.

Individuals with Hyperlipidemia Managed with Lipid-Lowering Therapy

Clinical Context and Test Purpose

The purpose of nontraditional cardiac biomarker testing in individuals with hyperlipidemia managed with lipid-lowering therapy is to inform a decision to proceed with appropriate treatment .

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with hyperlipidemia managed with lipid-lowering therapy.

Interventions

The therapy being considered is nontraditional cardiac biomarker testing.

Comparators

Comparators of interest include routine care without biomarker testing.

Outcomes

The general outcomes of interest are OS, change in disease status, morbid events, and medication use.

Study Selection Criteria

Methodologically credible studies were selected using the following principles:

- The study population represents the population of interest. Eligibility and selection are described.
- The test is compared with a credible reference standard.
- If the test is intended to replace or be an adjunct to an existing test; it should also be compared with that test.
- Studies should report sensitivity, specificity, and predictive values. Studies that completely report true- and false-positive results are ideal. Studies reporting other measures (eg, receiver operating characteristic, area under operating characteristic, c-statistic, likelihood ratios) may be included but are less informative.
- Studies should also report reclassification of the diagnostic or risk category.

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Apolipoprotein B

Systematic Reviews

A number of RCTs of statin therapy have examined the change in apo B on-treatment in relation to clinical CAD outcomes and assessed whether apo B predicted outcomes better than LDL-C. Boekholdt et al (2012) published a patient-level meta-analysis of on-treatment levels of traditional and nontraditional lipids as a measure of residual risk.[98,] Eight studies enrolling 62,154 participants were included. The aHR for each 1 SD increase in apo B was 1.14 (95% CI, 1.11 to 1.18), which did not differ significantly from LDL-C (aHR, 1.13; 95% CI, 1.10 to 1.17; p=.21). The aHR for HDL-C was 1.16 (95% CI, 1.12 to 1.19), which was significantly greater than LDL-C or apo B (p=.002). In a subsequent report from this meta-analysis, Boekholdt et al (2014) evaluated the LDL-C, non-HDL-C, and apo B levels of 38,153 patients allocated to the statin therapy groups.[99,] Despite statin therapy, reductions in levels of LDL-C, non-HDL-C, and apo B from baseline to 1 year showed large interindividual variations.

Randomized Controlled Trials

Ballantyne et al (2013) reported on a post hoc analysis of 682 patients with acute coronary syndrome from the randomized, phase 3 Limiting Undertreatment of Lipids in Acute coronary syndrome with Rosuvastatin trial.[100,] The Limiting Undertreatment of Lipids in Acute coronary syndrome with Rosuvastatin subgroup analysis examined apo B in relation to LDL-C and non-HDL-C under intensive statin therapy with rosuvastatin or atorvastatin. The treatment target level for apo B of 80 mg/dL correlated with an LDL-C level of 90 mg/dL and a non-HDL-C level of 110 mg/dL at baseline and with an LDL-C of 74 mg/dL and a non-HDL-C of 92 mg/dL with statin therapy. Independent of triglyceride status, non-HDL-C was found to have a stronger correlation with apo B than with LDL-C and could be an adequate surrogate for apo B during statin therapy.

The AFCAPS/TexCAPS trial (2000) evaluated lipid parameters among 6605 men and women with average LDL-C and low HDL-C levels who were randomized to lovastatin or placebo.[33,] Baseline LDL-C, HDL-C, and apo B levels 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.

In the Long-term Intervention with Pravastatin in Ischemic Disease trial (2002), the relation between on-treatment apo B levels and clinical outcomes was examined in 9140 patients randomized to pravastatin or placebo and followed for a mean of 6.1 years.[34,]The aHR for apo B levels (2.10; 95% CI, 1.21 to 3.64 ; p=.008) was higher than that for LDL-C (1.20; 95% CI, 1.00 to 1.45; p=.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 (2008) combined data from 2 RCTs, the Treating to New Targets (TNT) and Incremental Decrease in End Points Through Aggressive Lipid Lowering trials, to compare the relation between response to lipids, apo B levels, and other lipid measures.[35,] The analysis included 18,889 patients with established coronary disease randomized 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<.001), while LDL level was not. Similarly, 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 TC/HDL-C ratio was not. In another publication that reported on the TNT study (2012), the on-treatment apo B level was also a significant predictor of future events (aHR, 1.19; 95% CI, 1.11 to 1.28).[36,] 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 (2012) measured on-treatment lipid levels to assess the prediction of residual risk while on statin therapy.[101,] Using data from the JUPITER trial, on-treatment levels of LDL-C, non-HDL-C, high-sensitivity CRP, apo B, and apo AI were used to predict subsequent cardiovascular events. The HRs for cardiovascular events were similar among the lipid measures, ranging from 1.22 to 1.31, with no significant differences between them. The residual risk declined overall with a decreasing level of LDL-C, with the lowest risk seen in subjects achieving an LDL-C level of less than 70 mg/dL.

Subsection Summary: Apolipoprotein B

As a marker of response to cholesterol-lowering treatment, apo B may be more accurate than LDL-C and may provide a better measure of the adequacy of anti-lipid therapy than 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. Currently, it is not 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 risk assessment and/or treatment response. The most widely used risk

assessment models (e.g., the Framingham prediction model) and the most widely used treatment guidelines (e.g., 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 AI measurements to routine clinical care.

Apolipoprotein AI

Randomized Controlled Trials

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. For example, in the Kastelein et al (2008) study (described above), authors combined data from 2 RCTs, the TNT, and the Incremental Decrease in End Points Through Aggressive Lipid Lowering trials, to compare the relation between response to lipids, apo B/apo AI ratio, and other lipid measures.[35,] The apo B/apo AI ratio was a significant predictor of events (HR, 1.24; 95% CI, 1.17 to 1.32) while the TC/HDL-C was not.

The Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in MI (PROVE-IT TIMI) study (2009) randomized 4162 patients with an acute coronary syndrome to standard statin therapy or intensive statin therapy.[102,] While the on-treatment apo B/apo AI ratio was a significant predictor of cardiac events (HR for each 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 TC/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 AI have demonstrated plaque regression in a small number of patients with the acute coronary syndrome.[103,] Based on this research, there has been an interest in developing synthetic apo AI mimetic proteins, and such agents are in the drug development stage. These types of agents would likely target patients with residual cardiac risk following maximal statin therapy, especially patients with low HDL levels.

Subsection Summary: Apolipoprotein AI

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 as 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. Apolipoprotein AI has not been incorporated into quantitative risk assessment models or treatment guidelines that can be used in clinical practice (e.g., the ATP III).[11,] The ATP III practice guidelines continue to tie clinical decision making to conventional lipid measures, such as 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

Randomized Controlled Trials

Apolipoprotein 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 have suggested that patients with an apo e4 allele may respond better to diet-modification strategies.[104,105,] Other studies have suggested that response to statin therapy may vary by *APOE* genotype and that the e2 allele indicates greater responsiveness to statins.[104,106,]

Chiodini et al (2007) examined the differential response to statin therapy by *APOE* genotype in a reanalysis of data from the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto-Prevenzione (GISSI-P) study.[107,] The GISSI-P study was an RCT comparing pravastatin with placebo in 3304 Italian patients with previous MI. Patients with the apo e4 allele treated with statins had a better treatment response as evidenced by lower overall mortality rates (1.85% vs. 5.28%,

respectively; p=.023), while there was no difference in mortality rates for patients not treated with statins (2.81% vs. 3.67%, respectively; p=.21). This study corroborated results reported previously but did not provide evidence that changes in treatment should be made as a result of the *APOE* genotype.

Observational Studies

Other studies have evaluated *APOE* genetic status as a predictor of response to lipid-lowering therapy. Donnelly et al (2008) reported on 1383 patients treated with statins from the Genetics of Diabetes Audit and Research in Tayside, Scotland (Go-DARTS) database.[108,] Researchers reported on final LDL levels and percentages of patients achieving target LDL by *APOE* genetic status. LDL levels following treatment were lower for patients who were homozygous for apo e2 (0.6 mmol/L) than for patients homozygous for apo e4 (1.7 mmol/L; p<.001). All patients who were homozygous for apo e2 reached their target LDL level compared with 68% of patients homozygous for apo e4 (p<.001). Vossen et al (2008) evaluated response to diet and statin therapy by apo E status in 981 patients with CAD who were enrolled in a cardiac rehabilitation program.[109,] They reported that patients with an apo e4 allele were more responsive to 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 *APOE* genetic status, with 30% to 47% of the variation in response to treatment explained by baseline LDL, compared with only 1% of the variation explained by *APOE* status.

Subsection Summary: Apolipoprotein E

The evidence on the response to treatment indicates that *APOE* genotype may be a predictor of response to statins and may allow clinicians to better guage a patient's chance of successful treatment, although not all studies have consistently reported this relation. At present, it is unclear how this type of information would 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 would choose alternative therapies, even in the presence of an *APOE* phenotype that indicates a diminished response.

None of the available evidence has provided adequate data to establish that the *APOE* genotype or phenotype improves outcomes when used in clinical care.

Low-Density Lipoprotein Subclass and Low-Density Lipoprotein Particle Size and Concentration

Patients with subclass pattern B have been reported to respond more favorably to diet therapy than those with subclass pattern A.[110,] Subclass pattern B has also been shown to respond more favorably to gemfibrozil and niacin, with a shift from small, dense LDL particles to larger LDL particles. While statin drugs lower the overall concentration of LDL-C, there is no shift to the larger LDL particles.

Randomized and Nonrandomized Controlled Trials

Superko et al (2005) reported that the response to gemfibrozil differed in patients who had LDL subclass A compared with those who had LDL subclass B.[111,] There was a greater reduction in the small, LDL levels for patients with subclass B, but this did not correlate with clinical outcomes. Another study has reported that atorvastatin treatment led to an increase in mean LDL size, while pravastatin treatment led to a decrease in LDL size.[112,]

Various studies have generally confirmed that small, dense LDL is impacted preferentially by fibrate treatment[113,114,115,] and possibly also by statin therapy.[113,115,] However, none demonstrated that preferentially targeting small, dense LDL leads to improved outcomes, compared with standard LDL targets widely used in clinical care.

Several trials with angiographic outcomes have examined the change in LDL particle size in relation to the angiographic progression of CAD. The 1996 Stanford Coronary Risk Intervention Project trial studied the relation between small, dense LDL and the benefit of diet, counseling, and drug therapy

in patients with CAD, as identified by initial coronary angiogram.[116,] Patients with subclass pattern B showed a significantly greater reduction in CAD progression than those with subclass pattern A. The 1990 Familial Atherosclerosis Treatment Study randomized patients from families with premature CAD and elevated apo B levels.[117,] Change in LDL particle size correlated significantly with the angiographic progression of CAD in this study.

Fewer studies have evaluated clinical outcomes in relation to LDL particle size. In the 2001 Cholesterol and Recurrent Events trial, survivors of MI with normal cholesterol levels were randomized to lipid-lowering therapy or placebo.[118,] A post hoc analysis from this trial failed to demonstrate a correlation between change in particle size and treatment benefit.

Subsection Summary: Low-Density Lipoprotein Subclass and Low-Density Lipoprotein Particle Size and Concentration

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 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.

Lipoprotein(a)

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 Lp(a) measurements as an intermediate outcome. 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.[119,120,]

Subsection Summary: Lipoprotein(a)

There is considerable uncertainty regarding the clinical utility of measuring Lp(a), specifically how knowledge of Lp(a) levels can be used in the clinical care of patients 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 insufficiently related to the impact on clinical outcomes.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Section Summary: Individuals with Hyperlipidemia Managed with Lipid-Lowering Therapy

Evidence for individuals with hyperlipidemia managed with lipid-lowering therapy who receive nontraditional cardiac biomarker testing includes analyses of the intervention arm(s) of lipid-lowering medication trials. In particular, apo B, apo AI, and apo E have been evaluated as markers of lipid-lowering treatment success, and evidence from the intervention arms of several RCTs 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.

Lipoprotein-Associated Phospholipase A_2 and Cardiovascular Risk

A large body of literature has accumulated on the utility of risk factors in the prediction of future cardiac events. The evidence assessed for this review consists of several systematic reviews, of prospective cohort studies that have evaluated the association between lipoprotein-associated phospholipase A_2 (Lp-PLA_2) and cardiovascular outcomes.

The National Cholesterol Education Program ATP-III guidelines have indicated that to determine the clinical significance of Lp-PLA_2, the emerging risk factors should be evaluated against the following criteria[121]:

- 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 measurements must be widely available, well-standardized, inexpensive, have accepted population reference values, and be relatively stable biologically.
- Preferably, but not necessarily, modification of the risk factor in clinical trials will have shown a reduction in risk.

Clinical Context and Test Purpose

The purpose of Lp-PLA_2 testing in patients who have a risk of CVD is to inform, improve patient stratification using risk prediction models that alter management decisions and improve health outcomes.

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals at risk for CAD.

Interventions

The relevant intervention of interest is testing for Lp-PLA_2 as a biomarker of CAD.

Comparators

The following practice is currently being used to manage CAD risk: standard assessment of cardiovascular risk.

Outcomes

The primary outcomes of interest are the development of CVD such as CAD, stroke, and mortality.

The development of CVD typically occurs over many years or decades.

Study Selection Criteria

For the evaluation of clinical validity of Lp-PLA_2 testing, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described

Clinically Valid

Lipoprotein-Associated Phospholipase A_2 as a Predictor of Coronary Artery Disease

Results of numerous, large-scale observational studies have examined whether Lp-PLA_2 is an independent risk factor for CAD. These observational studies have been analyzed in several

systematic reviews.[18,122,123,] The largest, conducted by The Emerging Risk Factors Collaboration (2012), included 37 cohort studies and performed a patient-level meta-analysis of the association between novel lipid risk factors and cardiovascular risk over a median follow-up of 10.4 years in patients without CVD.[18,] The review found Lp-PLA_2 was an independent risk factor for cardiovascular events with an HR of 1.12 (95% CI, 1.09 to 1.21) for each 1 standard deviation increase in Lp-PLA_2 activity based on 11 studies (N=32,075). However, there was no significant improvement in risk reclassification following the addition of Lp-PLA_2 to the reclassification model, with a net reclassification change of 0.21 (95% CI, -0.45 to 0.86).

Two other systematic reviews reported similar results. One review of 32 studies (N=79,036) found for every 1 standard deviation increase in Lp-PLA_2 levels, the relative risk was 1.10 (95% CI, 1.04 to 1.17) for CAD, 1.08 (95% CI, 0.97 to 1.20) for stroke, and 1.16 (95% CI, 1.09 to 1.24) for vascular death, following adjustment for traditional risk factors. There was also a significant association between Lp-PLA_2 levels and nonvascular deaths (RR, 1.10; 95% CI, 1.04 to 1.17).[122,] The second, smaller review (14 studies, N=20,549) reported a pooled OR of 1.60 (95% CI, 1.36 to 1.89), adjusted for traditional cardiac risk factors, for the development of future cardiac events with elevated Lp-PLA_2 levels.[123,]

Section Summary: Clinically Valid

Several large meta-analyses found consistent evidence that Lp-PLA_2 level is an independent predictor of CAD. Based on these reviews, it is less clear the degree to which Lp-PLA_2 improves on existing CAD prediction models regarding clinically important magnitudes of reclassification.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

No studies were identified that assessed the clinical utility of Lp-PLA_2 test to define CAD risk.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Although studies have shown that Lp-PLA_2 level is an independent risk factor for CAD, clinical utility depends on whether the use of Lp-PLA_2 levels improves on existing models of CAD prediction, which then translates into differences in treatment that improve patient outcomes. Establishing improved outcomes compared with existing prediction models could be demonstrated with clinical trials, but the expected difference in outcomes would probably be so small that the sample size of the trial would be impractically large. Decision modeling is another approach to estimating differences in patient outcomes due to the improved reclassification of risk. A robust, validated model using Lp-PLA_2 levels to predict CAD outcomes is necessary to use the test to manage patients. No studies identified evaluated whether a testing strategy that uses Lp-PLA_2 levels improves health outcomes.

Section Summary: Clinically Useful

Changes in patient management that could potentially occur with a strategy using Lp-PLA_2 levels are not well-established. Studies that directly evaluate patient management changes and/or health outcome improvements are needed to determine whether the use of Lp-PLA_2 measurement has efficacy in CVD. Alternatively, robust decision modeling studies may demonstrate clinically important changes in health outcomes by incorporating Lp-PLA_2 levels into CAD prediction models. Groups

such as the American Heart Association have often incorporated results from decision models to inform their guidelines when the data underlying the models are robust. Incorporation of Lp-PLA_2 into decision models is necessary to demonstrate the potential clinical utility of the biomarker.

Cardiovascular Disease Risk Testing Panels

Clinical Context and Test Purpose

The purpose of CVD risk panel testing in individuals who have risk factors for CVD is to inform management and treatment decisions.

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with risk factors for CVD.

Interventions

The relevant intervention of interest is testing with CVD risk panels.

Comparators

The following practice is currently being used to manage those at risk for CVD: management of clinical risk factors with or without simple lipid testing.

Outcomes

The beneficial outcomes of interest are decreases in morbidity and mortality from CVD.
The development of CVD occurs over many years and manifests as CHD, CVD, or peripheral arterial disease. The timing for measuring outcomes can range from 5 to 10 years.

Study Selection Criteria

For the evaluation of the clinical validity of the tests, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described
- Included a validation cohort separate from the development cohort.

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence

Association Between Single Risk Markers and Cardiovascular Disease Risk

Systematic Reviews

There is a large evidence base on the association between individual risk markers and CVD risk. Many observational studies have established that individual risk markers are independent predictors of cardiac risk.[3,5,]

Antonopoulos et al (2022) conducted a meta-analysis to evaluate biomarkers of vascular inflammation for cardiovascular risk prognosis in stable patients without known CHD.[124,] Various biomarkers of vascular inflammation (such as CRP, interleukin-6 and tumor necrosis factor-alpha) were evaluated in the 39 studies (N=175,778) that were included. The primary composite endpoint was the difference in c-index with the use of inflammatory biomarkers for MACE and mortality. Vascular inflammation biomarkers provided added prognostic value for the composite endpoint and for

MACE only. However, limitations in the published literature included a lack of reporting on the net clinical benefit, cost-effectiveness of such biomarkers in clinical practice, and other metrics of improvement of risk stratification.

Van Holten et al (2013) conducted a systematic review of meta-analyses of prospective studies evaluating the association between serologic biomarkers and primary cardiovascular events (i.e., cardiovascular events and stroke in CVD-naive populations) and secondary cardiovascular events (i.e., cardiovascular events and stroke in populations with a history of CVD).[14,] The final data synthesis included 85 studies published from 1988 to 2011. Sixty-five meta-analyses reported biomarkers' association with primary cardiovascular events and 43 reported associations with secondary cardiovascular events. Eighteen meta-analyses reported biomarkers' association with ischemic stroke in patients with a history of CVD. Only 2 meta-analyses that reported associations with ischemic stroke in patients with no history of CVD were identified, and results were not reported. The CVD risks for markers with the strongest associations are summarized in Table 4.

Table 4. Serum Biomarkers and Cardiovascular Risk

Marker	RR, HR, or OR	95% Confidence Interval
Prediction of CV events in patients with no history of CVD		
C-reactive protein	2.43 (RR)	2.10 to 2.83
Fibrinogen	2.33 (HR)	1.91 to 2.84
Cholesterol	0.44 (HR)	0.42 to 0.48
Apo B	1.99 (RR)	1.65 to 2.39
Apo A: Apo B ratio	1.86 (RR)	1.55 to 2.22
HDL	1.83 (HR)	1.65 to 2.03
Vitamin D	1.83 (HR)	1.19 to 2.80
Prediction of CV events in patients with a history of CVD		
cTn I and T	9.39 (OR)	6.46 to 13.67
High-sensitivity C-reactive protein	5.65 (OR)	1.71 to 18.73
Creatinine	3.98 (HR)	3.02 to 5.24
Cystatin C	2.62 (RR)	2.05 to 3.37
Prediction of ischemic stroke in patients with a history of CVD		
Fibrinogen	1.75 (HR)	1.55 to 1.98
Uric acid	1.47 (RR)	1.19 to 1.76

Adapted from van Holten et al (2013)[14,]
Apo: apolipoprotein; CI, confidence interval; cTn: cardiac troponin; CV: cardiovascular; CVD: cardiovascular disease; HDL: high-density lipoprotein; HR; hazard ratio; OR: odds ratio; RR: relative risk.

Prospective and Retrospective Studies

Since the publication of the van Holten et al (2013) review, multiple studies have reported on the associations between various risk markers and CVD outcomes. Representative examples of reported associations include: endothelin-1 in predicting mortality in patients with heart failure with reduced ejection fraction[125,]; troponin and NT-proBNP in predicting CVD-related death[126,127,]; growth differentiation factor and interleukin 6 with CVD- and non-CVD-related death[126,], mid-regional pro-atrial natriuretic peptide and C-terminal pro-endothelin-1 with morbidity and mortality after cardiac surgery [128,], and triglyceride-glucose index with the incidence of acute coronary syndrome.[129,]
Mohebi et al (2023) conducted a review of data from the Catheter Sampled Blood Archive in Cardiovascular Diseases (CASABLANCA) cohort study to identify a panel of biomarkers to help stratify patient risk for CV events within 2 years of coronary angiography.[130,] All patients in the study (n=446) had chronic kidney disease (stage 1 to 2, 84.8%; stage 3 to 5, 15.2%). Monte Carlo simulation was used to identify a prognostic panel of biomarkers, which consisted of NT-proBNP, kidney injury molecule-1, osteopontin, and tissue inhibitor of matrix metalloproteinase-1. The panel had a C-statistic for predicting CV events of 0.77 (95% CI, 0.72 to 0.82). Among patients with stage 1 to 2 chronic kidney disease, the HR for CV events was 2.82 (95% CI, 1.53 to 5.22) in patients with higher cardiovascular risk compared to lower cardiovascular risk. In patients with stage 3 to 5 chronic kidney disease, the HR was 8.32 (95% CI, 1.12 to 61.76) in patients with higher CV risk compared to lower CV risk.

Safo et al (2023) derived a protein biomarker risk score to predict CVD in patients with HIV.[131] The risk score was derived from 4 trials conducted by the International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) and included the following 8 proteins: FAM3B, integrin α11, interleukin-6, hepatocyte growth factor, C-C motif chemokine 25, gastrotropin, platelet-activating factor acetylhydrolase, and secretoglobin family 3A member. After adjusting for CVD at baseline and HIV-related factors, the protein score was associated with an increased risk of CVD (OR, 2.17; 95% CI, 1.58 to 2.99).

Wallentin et al (2021) analyzed data in a subset of patients with chronic CHD from the Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) trial to assess the association between various CV and inflammatory biomarkers and CV death; patients in the STABILITY trial had a median follow-up time of 3.7 years.[132] Biomarkers were compared between patients who experienced CV death (n=605) and those who did not experience CV death (n=2788). Another prospective observational study (the Ludwigshafen Risk and Cardiovascular Health [LURIC] study) was used for replication. This study followed a cohort of 3316 patients scheduled for coronary angiography over a period of 12 years to assess cardiovascular mortality. Both studies included patients with a median age of 65 years and 20% smokers; the STABILITY trial included 82% males, 70% with hypertension, and 39% with diabetes while the LURIC trial had 76% males, 78% with hypertension, and 30% with diabetes. Unadjusted and adjusted Cox regression analyses showed that NT-proBNP (HR for 1 standard deviation [SD] increase of the log scale of the distribution of the biomarker in the replication cohort, 2.079; 95% CI, 1.799 to 2.402) and high-sensitivity troponin T (HR, 1.715; 95% CI, 1.491 to 1.973) had the highest prognostic values for CV death.

Wuopio et al (2018) analyzed 10-year data from the CLARICOR trial in Denmark to investigate the association between serum levels of cathepsin B and S and CV risk and mortality in patients with stable CHD.[133] The researchers used the placebo group (n=1998) as a discovery sample and the treatment group (n=1979) as a replication sample. A multivariable Cox regression model was used to adjust for risk factors and other variables. Analysis showed that cathepsin B was associated with an increased risk of CV events and mortality ($p<.001$ for both groups), but cathepsin S was not ($p>.45$). Limitations included unknown generalizability to patients with acute symptoms, other ethnic groups, and those unlikely to volunteer for such trials. In another evaluation involving the placebo group of the CLARICOR trial (n=1998), Winkel et al (2020) evaluated whether 12 novel circulating biomarkers (NT-proBNP, high-sensitive assay cardiac troponin T, YKL40, osteoprotegerin, pregnancy-associated plasma protein A, cathepsin B, cathepsin S, endostatin, soluble tumor necrosis factors 1 and 2, calprotectin, and neutrophil gelatins-associated lipocalin) when added to "standard predictors" (eg, age, smoking, plasma lipids) improved the 10-year prediction of CV events and mortality in patients with stable CHD.[134] Results of the analysis revealed that the overall contribution of these novel biomarkers to all-cause death and composite CV outcome predictions was minimal. Two of the 12 biomarkers (calprotectin and cathepsin S) were not associated with the outcomes, not even as single predictors. The addition of the 10 remaining biomarkers to the "standard predictors" only increased the correct all-cause death predictions from 83.4% to 84.7% and the composite outcome predictions from 68.4% to 69.7%.

Welsh et al (2017) analyzed data from the Reduction of Events by Darbepoetin Alfa in Heart Failure (RED-HF) drug trial to assess the prognostic value of emerging biomarkers in CVD screening.[135] A panel of several biomarkers was measured at randomization in 1853 participants with complete data, and the relation between these biomarkers and a primary composite endpoint of heart failure hospitalization or CV death over 28 months of follow-up (n=834) was evaluated using Cox proportional hazards regression. Analysis showed that NT-proBNP (HR, 3.96) and high-sensitivity troponin T (HR, 3.09) far outperformed other emerging biomarkers studied for predicting adverse CV outcomes. Limitations included the homogenous sample from the trial cohort and regional differences.

Harari et al (2017) conducted a prospective cohort study analyzing the association between non-HDL-C levels and CVD mortality in a long-term follow-up of 4832 men drawn from the Cardiovascular Occupational Risk Factor Determination in Israel Study.[136,] Patients were between the ages of 20 and 70 years (mean age, 42.1 years at baseline); all completed multiple questionnaires that evaluated medical history and possible risk factors for CVD, in addition to blood tests. Before adjusting for potential confounders, a positive association was found between several comparator cholesterol categories (simple lipids including TC, triglycerides, and HDL-C) and all-cause or CVD mortality; however, in multivariate analysis, many of these associations were no longer statistically significant. For one of the primary outcomes (the efficacy of non-HDL-C in predicting CVD mortality), after adjusting for the known risk factors, results were statistically significant, with an association between non-HDL-C levels greater than 190 mg/dL and risk of mortality from CVD (HR, 1.80; 95% CI, 1.10 to 2.95; p=.020). Another primary outcome was the prediction value of non-HDL for all-cause mortality. For this outcome, the association between all levels of non-HDL-C were statistically insignificant after adjusting for potential confounders (for 130 to 159 mg/dL, p=.882; 160 to 189 mg/dL, p=.611; ≥190 mg/dL, p=.464). Likewise, the association between simple lipids and all-cause mortality was not statistically significant after adjusting for confounders. The authors also acknowledged that the association between CVD mortality and higher non-HDL-C levels (≥190 mg/dL) was not statistically significant when adjusting for LDL-C (HR, 2.39; 95% CI, 0.92 to 6.13; p=.073), but concluded that given the trends in p-values, non-HDL-C levels appeared superior at predicting mortality compared with simple lipid testing.

Kunutsor et al (2016) published both a primary analysis and meta-analysis of current studies evaluating the association between levels of paraoxonase-1 (PON-1) and CVD risk; for all analyses, the primary endpoint was first-onset CVD.[137,] Of 6902 patients drawn from the Prevention of Renal and Vascular End-stage Disease (PREVEND) study, the mean age was 48 years, and 3321 (48%) of the patients were men; for the meta-analysis, researchers used data from 6 studies (N=15,064). The authors noted that PON-1 activity showed a log-linear association with CVD risk, but compared the independence of PON-1 with that of HDL-C. In a model adjusted for known risk factors and confounding elements, PON-1 had an HR of 0.93 (95% CI, 0.86 to 0.99; p=.037); comparatively, HDL-C showed a stronger association with risk of CVD given the same adjustments (HR, 0.84; 95% CI, 0.76 to 0.94; p=.002). Also, the HR for PON-1 was no longer statistically significant when the model accounted for HDL-C (0.95; 95% CI, 0.88 to 1.02; p=.153), suggesting that the link between PON-1 and HDL-C inhibits the independence of PON-1 as a risk marker. Secondary endpoints were CHD and stroke. For CHD, as with CV events, HRs for PON-1 were not statistically significant when fully adjusted for confounders (p=.058) and HDL-C (p=.471), compared with a strong association between HDL-C and CHD (HR, 0.67; 95% CI, 0.57 to 0.78; p<.001). The meta-analysis was limited by considerable heterogeneity between studies, but resulted in a pooled relative risk of 0.87 (95% CI, 0.80 to 0.96; p=.005), reported as the CV event per 1 SD increase in PON-1 values. Acknowledging the link between PON-1 and HDL-C as risk markers, the authors concluded that PON-1 added "no significant improvement in CVD risk assessment beyond conventional CVD risk factors."

Risk Markers and Cardiovascular Disease Risk Reclassification

Other studies have demonstrated that risk markers can be used to reclassify patients into different risk categories. Helfand et al (2009) reported on a summary of 9 systematic reviews evaluating novel risk factors' association with CHD.[3,] Of the laboratory risk factors evaluated, CRP, homocysteine, and lipoprotein (a) were independent predictors of major CHD events when added to the Framingham Risk Score (FRS). However, none of the available systematic reviews evaluated the effect of each novel risk factor on risk-classification among patients classified as intermediate risk by the FRS. In a 2012 study of 165,544 patients without baseline CVD enrolled in 37 prospective cohorts, the addition of individual novel lipid-related risk factors to conventional risk-classification models resulted in net reclassification improvements of less than 1% with the addition of each marker.[18,]

Association Between Multimarker Panels and Cardiovascular Disease Risk

A more limited body of literature has evaluated the association between panels of markers and CVD risk and/or the reclassification of patients into different risk categories.

Keller et al (2017) conducted a case-control study of the prognostic ability of a panel of 5 micro-RNAs (miR-34a, miR-223, miR-378, miR-499, miR-133), using 2 cohorts with patients randomly selected from previous studies. The combined primary outcome was overall mortality and CV events.[138,] In the derivation cohort, 21 of 178 patients experienced a CV event and/or death within 5 years. In the validation cohort, which excluded patients with a history of CVD, 64 of 129 patients died during a 12-year follow-up. While the individual micro-RNAs lacked a significant association with the outcome, the panel as a whole improved both prognostic and predictive value for overall mortality, particularly when adjusted for FRS variables (HR, 2.89; 95% CI, 1.32 to 6.33; p=.008). For the derivation cohort, the investigators reported an increase in the AUC from 0.77 to 0.85 with the addition of the miR panel in predicting mortality risk within 5 years (p=.039). This improvement was confirmed by a net reclassification index (NRI) of 0.42 in the validation cohort (p=.014). The authors reported that the C index was statistically unaffected by the miR panel, but that the miR panel was significantly associated with mortality in the validation cohort (HR, 1.31; 95% CI, 1.03 to 1.66; p=.03).

A prospective cohort study by de Lemos et al (2017) evaluated a panel of 5 biomarker tests to develop a composite score to predict CVD risk.[139,] The 2 cohorts were drawn from the Multi-Ethnic Study of Atherosclerosis (MESA) and the Dallas Heart Study (DHS): from MESA, 3112 (47%) patients were men; and from DHS, 969 (44%) of the patients were men, none of whom had prevalent CVD at baseline. Each test had its own prespecified level of abnormality: a 12-lead electrocardiogram measured the presence or absence of left ventricular hypertrophy. Additional tests measured for coronary artery calcium levels greater than 10 units, NT-proBNP levels of 100 pg/mL or more, high-sensitivity cardiac troponin levels of 5 ng/L or more, and high-sensitivity CRP (hs-CRP) levels of 3 mg/L or more. Test data were analyzed as categorical and continuous variables, and included models with and without all 5 test results. In all models for MESA, there was an independent association between the tests and the primary endpoint (global CVD). There was no association between hs-CRP and the primary outcome in the DHS cohort, between hs-CRP and a secondary outcome (atherosclerotic CVD) in the MESA cohort, or between hs-CRP and high-sensitivity cardiac troponin and atherosclerotic CVD in the DHS cohort. In MESA, the C statistic for the primary outcome increased from 0.73 when adjusted for variables alone to 0.786 when adjusted for individual test results (p<.001), and the DHS cohort showed a similar significant improvement (0.832 to 0.850; p<.01). The category-free NRI for both cohorts were as follows: MESA NRI, 0.473 (95% CI, 0.383 to 0.563); and DHS NRI, 0.261 (95% CI, 0.052 to 0.470). Based on the results from the 5 tests, the authors assigned each patient a risk score, which they suggested could aid caregivers in identifying patients who need specific treatment or changes in preventive management.

Greisenegger et al (2015) evaluated the association between a panel of biomarkers and mortality after a transient ischemic attack and minor ischemic stroke.[140,] The study population included 929 patients who were enrolled from 2002 to 2007 and followed until 2013. Fifteen potential risk markers were prospectively measured (interleukin 6, CRP, neutrophil-gelatinase-associated lipocalin, soluble tumor necrosis factor α receptor-1, thrombomodulin, fibrinogen, von Willebrand factor, P-selectin, protein Z, D-dimer, antiphosphorylcholin, NT-proBNP, heart-type fatty acid-binding protein, neuron-specific enolase, and brain-derived neurotrophic factor). None of the biomarkers were predictive of nonfatal ischemic stroke or myocardial infarction (MI). Six factors were individually associated with CVD death, of which the 4 with the strongest association (von Willebrand factor, heart-type fatty acid-binding protein, NT-proBNP, and soluble tumor necrosis factor α receptor-1) were entered into a predictive model. The independent contribution of the 4 biomarkers taken together added more prognostic information than the established clinical risk factors used in a conventional model (clinical risk factors , p=.002; 4 biomarkers , p<.001).

Cho et al (2015) reported on the impact of 6 biomarkers (hs-CRP; interleukin 6; receptor for advanced glycation end products; lipoprotein-associated phospholipase A_2; adiponectin; regulated on activation, normal T cell expressed and secreted) on CVD risk-classification in a case-control study of 503 patients with coronary artery disease and 503 healthy controls.[141] The addition of the 6 novel biomarkers to the multivariable risk prediction model led to an improvement in the C statistic (0.953 vs. 0.937 ; p<.001). However, the performance of the model in a cohort not enriched with coronary artery disease patients is unknown.

Wilsgaard et al (2015) evaluated 51 protein biomarkers for association with a risk of incident MI with the goal of developing a clinically significant risk model that would add information to conventional risk models.[142] Patients were drawn from a population-based cohort study to form a case-control study, with 419 cases who experienced a first-ever MI within the 10-year follow-up and 398 controls randomly selected from participants who had no MI during the follow-up. Fifty-one markers were selected for evaluation based on previously reported associations and the availability of immunoassay techniques and passage of internal quality controls. Seventeen markers were predictive of MI after adjustment for traditional CVD risk factors. By adding risk markers back into the traditional risk factor-based model, the authors determined that a composite of apo B/apo AI, plasma kallikrein, lipoprotein (a), and matrix metalloproteinase 9 increased the model's area under the receiver operating curve by 0.027, with an NRI of 9%.

Guarrera et al (2015) evaluated DNA methylation profiles and Long Interspersed Nuclear Element 1 (LINE-1) hypomethylation in the prediction of MI, analyzing data from 609 cases and 554 controls drawn from the Italian European Prospective Investigation into Cancer and Nutrition study (EPICOR), and the Dutch EPIC study (EPIC-NL).[143] Rather than analyze single 5′-C-phosphate-G-3′ sites for their association with CVD, the authors focused on differentially methylated regions, as well as LINE-1 methylation profiles, adjusting models to account for their addition to traditional risk factors. A cluster of 15, 5′-C-phosphate-G-3′ sites, was statistically significant in both cohorts; the region was in exon 1 of the zinc finger and BTB domain, containing the protein 12 gene (*ZBTB12*), and showed hypomethylation comparable between EPICOR cases and controls (effect size, -0.019; 95% CI, -0.03 to -0.01; p=1.94 x 10^{-7} ; *Q*=0.005). Although the association was not statistically significant for women in the EPICOR cohort, the EPIC-NL cohort showed significant hypomethylation in the *ZBTB12* region between cases and controls as a whole (effect size, -0.013; 95% CI, -0.02 to -0.005; p<.001), as well as for male (effect size, -0.014; 95% CI, -0.03 to -0.001; p=.034) and female subgroups (effect size, -0.012; 95% CI, -0.02 to -0.004; p=.006). There was also a significant association between LINE-1 hypomethylation in EPICOR cases versus controls (effect size, -0.511; 95% CI, -0.80 to -0.22; p<.001), and this association held for the male subgroup (effect size, -0.520; 95% CI, -0.87 to -0.17; p=.004) but not in the female subgroup (effect size, -0.496; 95% CI, -1.12 to -0.13; p=.12). Secondary endpoints involved comparing the risk prediction for MI in the cumulative DNA methylation profile of LINE-1 sequences with that of traditional risk factors alone. While the association between LINE-1 and MI was significant for men in the EPIC-NL cohort (overall response, 1.95; 95% CI, 1.02 to 3.71; p=.043, reference group above the median), the association was not significant for women in this same cohort (overall response, 1.05; 95% CI, 0.65 to 1.67; p=.850). When the model included both traditional risk factors and the DNA methylation profile, NRI and integrated discrimination improvement measures were statistically significant, compared with risk factors alone. In the EPIC-NL cohort, NRI and integrated discrimination improvement among men were 0.47 (95% CI, 0.19 to 0.76; p=.001) and 0.04 (95% CI, 0.01 to 0.08; p=.004), respectively; among women, they were 0.23 (95% CI, 0.02 to 0.43; p=.034) and 0.03 (95% CI, 0.01 to 0.05; p=.001), respectively.

Association Between Multimarker Panels and Wellness

The preponderance of the literature on CVD risk panels have focused on the risk of specific events related to CVD (e.g., stroke, MI) or on the development of CVD. With the development of panels that address "wellness" more broadly, studies were sought on the association between risk markers and measures of overall wellness or health. No empirical studies were identified. Lara et al (2015) reported the recommendations of the U.K. Medical Research Council to develop recommendations for a panel

of biomarkers for healthy aging.[144,] A variety of markers, some laboratory-based, associated with the physical capability and physiologic, cognitive, endocrine, immune, and sensory functions were proposed.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy or testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

While multiple risk factors have been individually associated with CVD, there is no convincing evidence that the addition of any individual risk marker, or combination of risk markers, leads to clinically meaningful changes in management that improve outcomes. In the available studies, improvements in risk prediction have generally been of a small magnitude, and/or have not been found to be associated with clinically meaningful management changes.[3,18,145,] Because of this uncertain impact on management, the clinical utility for any of the individual risk markers is either low or uncertain.

Moreover, the available evidence on individual risk markers is only of limited value in evaluating CVD risk panels. It is difficult to extrapolate the results of single risk factors to panels, given the variable composition of panels. Ideally, panels should be evaluated individually based on their impact on clinical decision making.

No published studies were identified that evaluated the use of commercially available CVD risk panels as risk prediction instruments in clinical care. Some studies have attempted to incorporate novel risk markers into an overall quantitative risk score,[28,146,] but these risk scores are not the same as CVD risk panels, which report the results of individual risk factors.

Furthermore, there are no standardized methods for combining multiple individual risk factors with each other, or with established risk prediction instruments such as the FRS. Therefore, there is a potential for both overestimation and underestimation of the true cardiac risk. This may lead to management decisions based on an inaccurate risk assessment.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of CV risk panel testing has not been established, a chain of evidence cannot be constructed to support the clinical utility of testing.

Section Summary: Cardiovascular Disease Risk Testing Panels

Many of the individual risk factors included in CVD risk panels are associated with an increased risk of CVD. However, it is not clear how the results of individual risk factors impact management changes, so it is also uncertain how the panels will impact management decisions. Given the lack of evidence for the clinical utility of any individual risk factor beyond simple lipid measures, it is unlikely that the use of CVD risk panels improves outcomes. Studies that have evaluated the clinical validity of panels of multiple markers have not assessed management changes that would occur as a result of testing or demonstrated improvements in outcomes.

Supplemental Information

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Practice Guidelines and Position Statements

Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

National Heart, Lung, and Blood Institute

In 2001, the National Heart, Lung, and Blood Institute's National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) issued a position statement.[11,] Apolipoprotein B (apo B), apolipoprotein AI (apo AI), lipid subclass, and lipoprotein(a) (Lp[a]) were listed as "emerging risk factors" for cardiovascular risk assessment, without specific recommendations for how these measures should be used in clinical practice. A 2004 update to these guidelines discussed the results of clinical trials of statin therapy.[147,] In 2013, the Institute published a systematic evidence review on managing blood cholesterol in adults.[148,] The review was used to develop joint guidelines by the American College of Cardiology (ACC) and American Heart Association (AHA) on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults (see below).[149,]

American College of Cardiology and American Heart Association

In 2013, the ACC and the AHA published guidelines on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk and the assessment of cardiovascular risk.[149,150,] 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 cholesterol (HDL-C) 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 the variables significantly improved discrimination for 10-year hard ASCVD risk prediction. The ACC and AHA recommended that further research using state-of-the-art statistical techniques (including net reclassification improvement and integrative discrimination index) examine the utility of novel biomarkers when added to these new pooled cohort equations in different populations and patient subgroups. The guidelines stated 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. Regarding newer risk markers after quantitative risk assessment, the guidelines stated the following: "If, after quantitative risk assessment, a risk-based treatment decision is uncertain, assessment of ≥1 of the following: family history, hs-CRP [high-sensitivity C-reactive protein], CAC [coronary artery calcium] score, or ABI [ankle-brachial index] may be considered to inform treatment decision-making" (class of recommendation IIb, level of evidence B). The guidelines did not recommend other novel cardiac risk factors or panels of cardiac risk factors.

The ACC/AHA (2019) guidelines on primary prevention of cardiovascular disease include information on appropriateness of Lp(a) level measurement stating "a relative indication for its measurement is family history of premature ASCVD. An Lp(a) ≥50 mg/dL or ≥125 nmol/L constitutes a risk-enhancing factor, especially at higher levels of Lp(a)."[151,] The guidelines also include recommendations for apo B measurement stating, "a relative indication for its measurement would be triglyceride ≥200 mg/dL. A level ≥130 mg/dL corresponds to an LDL-C >160 mg/dL and constitutes a risk-enhancing factor." Llipoprotein-associated phospholipase A_2 (Lp-PLA_2) testing was not mentioned in these guidelines, which was a change from 2010 guidelines.[5,] In their prior guideline, Lp-PLA_2 was given a IIb recommendation for assessing cardiovascular risk in intermediate-risk asymptomatic adults.

American Diabetes Association and American College of Cardiology Foundation

In 2008, a consensus statement from the American Diabetes Association and the ACC Foundation addressed lipoprotein management in patients with cardiometabolic risk.[152,] This statement included specific recommendations for incorporating apo B testing into clinical care for high-risk patients and recommended that, for patients with metabolic syndrome 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 and on the limitations of the clinical utility of nuclear magnetic resonance measurement of LDL particle number or size, including lack of widespread availability. The statement also noted 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.

The American Diabetes Association 2023 Standards of Care do not discuss the use of specific novel biomarkers for cardiovascular disease and risk management. [153,]

American Association of Clinical Endocrinologists and the American College of Endocrinology

In 2017, the American Association of Clinical Endocrinologists (AACE, now the American Association of Clinical Endocrinology) and the American College of Endocrinology (ACE) published joint guidelines on the management of dyslipidemia and the prevention of cardiovascular diseases.[154,] The guidelines recommended that, among patients with "triglyceride (TG) concentration of greater than 150 mg/dL or HDL-C concentration of less than 40 mg/dL, the apo B or the apo B to apo AI ratio may be useful in assessing residual risk in individuals at risk for ASCVD (even when the LDL-C levels are controlled)." The guidelines also recommended the measurement of Lp-PLA_2 as an additional indication of cardiovascular risk. Citing several studies in which Lp-PLA_2 was comparable with hs-CRP as a risk predictor, the guidelines accordingly recommended the use of Lp-PLA_2 data in situations requiring a more specific evaluation of the risk of ASCVD than is provided by hs-CRP.

In 2020, the AACE published an updated consensus statement on dyslipidemia and prevention of cardiovascular disease.[155,] They recommended measurement of Lp(a) in several patient populations including those with ASCVD, those with a family history of premature ASCVD and/or increased Lp(a), and individuals with a 10-year ASCVD risk of 10% of greater. Recommendations also included consideration of apo B or LDL particle measurement "based on individual patient clinical circumstances."

In 2022, the AACE published a guideline on comprehensive care plans in patients with diabetes.[156,] In addition to treatment targets for LDL-C and non-HDL-C, the guideline defines target apo B levels of <90 mg/dL, <80 mg/dL, or <70 mg/dL for patients with high, very high, and extreme risk of ASCVD. Patients receiving statins should undergo monitoring for these parameters (including apo B) every 6 to 12 weeks, and monitoring frequency can decrease after targets are achieved.

European Society of Cardiology/European Atherosclerosis Society

In 2019, the European Society of Cardiology and European Atherosclerosis Society published a guideline for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk.[157,] This guideline contains updated recommendations for lipid analyses for cardiovascular disease risk estimation. Beyond traditional lipid markers (i.e., total cholesterol, HDL, LDL, and triglycerides), the guideline recommends non-HDL-C "for risk assessment, particularly in people with high triglyceride levels, diabetes mellitus, obesity, or very low LDL-C levels" [Class I recommendation; Level C evidence (consensus of opinion of the experts and/or small studies, retrospective studies, registries)]. Apolipoprotein B is recommended "for risk assessment, particularly in people with high triglyceride levels, diabetes mellitus, obesity, metabolic syndrome, or very low LDL-C levels. It can be used as an alternative to LDL-C, if available, as the primary measurement for screening, diagnosis,

and management, and may be preferred over non-HDL-C in people with high triglyceride levels, diabetes mellitus, obesity, or very low LDL-C levels" [Class I recommendation; Level C evidence]. Additionally, the guideline states that Lp(a) measurement "should be considered at least once in each adult person's lifetime to identify those with very high inherited lipoprotein(a) levels > 180 mg/dL who may have a lifetime risk of atherosclerotic CVD equivalent to the risk associated with heterozygous familial hypercholesterolemia" and "should be considered in selected patients with a family history of premature CVD, and for reclassification in people who are borderline between moderate and high-risk" [Class IIa recommendation; Level C evidence].

In 2021, the European Society of Cardiology published a guideline on CVD prevention; however, the guideline did not recommend specific novel cardiac risk factors or panels of cardiac risk factors for the assessment of CVD risk.[158,] The guideline states that "main causal and modifiable ASCVD [atherosclerotic cardiovascular disease] risk factors are blood apolipoprotein-B-containing lipoproteins, high BP [blood pressure], cigarette smoking, and DM [diabetes mellitus]". The guideline also states that the ABI may be considered as a risk modifier in CVD risk assessment but the "routine collection of other potential modifiers, such as genetic risk scores, circulating or urinary biomarkers, or vascular tests or imaging methods (other than CAC scoring or carotid ultrasound for plaque determination), is not recommended."

National Lipid Association

National Lipid Association (NLA) recommendations for patient-centered management of dyslipidemia were published in 2015.[159,] These recommendations stated that non-HDL-C and LDL-C should be primary targets for therapy and that apo B is an optional, secondary target for therapy. The Association favored non-HDL-C over apo B because the former is universally available and because apo B has not consistently shown superiority in predicting ASCVD risk.

In 2018, the NLA published a guideline on the management of blood cholesterol in conjunction with 11 other organizations, which discussed the measurement of apo B and Lp(a).[160,] A triglyceride level ≥200 mg/dL was mentioned as a relative indication of apo B measurement. Relative indications for measurement of Lp(a) include family history of premature ASCVD or ASCVD without traditional risk factors.

In 2019, the NLA issued a scientific statement on the use of Lp(a), which notes that Lp(a) measurement "is reasonable" to refine risk assessment for ASCVD events in the following populations: patients with first-degree relatives with premature ASCVD (<55 years of age for men; <65 years of age for women), patients with premature ASCVD without traditional risk factors, patients with severe hypercholesterolemia (LDL-C ≥190 mg/dL) or familial hypercholesterolemia, and patients with very-high risk of ASCVD that may be candidates for proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor therapy.[161,] Additionally Lp(a) "may be reasonable" to measure in patients with the following: intermediate (7.5% to 19.9%) or borderline (5% to 7.4%) ASCVD risk when statin initiation is uncertain for primary prevention, inadequate response to LDL-C lowering therapy despite adherence, family history of elevated Lp(a), calcific valvular aortic stenosis, or recurrent or progressive ASCVD despite lipid-lowering therapy.

In 2021, the NLA issued a scientific statement on lipid measurements in cardiovascular disease including information on apo B, small dense LDL, and Lp(a).[162,] The authors refer to the 2019 statement for information on Lp(a), and they recommend that measurements of apo B and small dense LDL "may be reasonable at initial evaluation." Additionally, apo B measurement "is reasonable" for patients receiving lipid lowering therapy while small dense LDL measurement is "not recommended" for these patients.

National Institute for Health and Care Excellence

In 2023, the NICE updated its guidance on risk assessment and reduction, including lipid modification of CVD.[163,] The guidance recommended measuring a full lipid profile including total cholesterol, HDL,

non-HDL, and triglycerides before starting lipid-lowering therapy for primary prevention of CVD. The guidance also recommended measurement of total cholesterol, HDL, non-HDL, and triglycerides for primary and secondary prevention in people on high-intensity statins at 3 months of treatment, aiming for a 40% reduction in non-HDL. Nontraditional risk factors, including apo B, were not discussed as part of risk assessment or treatment targets.

U.S. Preventive Services Task Force Recommendations

The U.S. Preventive Services Task Force (2009) issued recommendations on the use of nontraditional risk factors for the assessment of coronary heart disease (CHD).[3,] The Task Force included Lp(a) in its summary statement: "The evidence is insufficient to assess the balance of benefits and harms of using the nontraditional risk factors discussed in this statement to screen asymptomatic men and women with no history of CHD to prevent CHD events."

The recommendation was updated in 2018 and came to the same conclusion: evidence is insufficient to assess the benefits and harms of novel testing methods to diagnose CVD. However, the nontraditional risk factors included in this recommendation were different than those in this evidence review.[164,]

Medicare National Coverage

There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Ongoing and Unpublished Clinical Trials

Some currently unpublished trials that might influence this review are listed in Table 5.

Table 5. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Unpublished			
NCT03599531	A Pilot Study to Evaluate the Utility of the SomaLogic CVD Secondary Risk Panel as a Tool to Stratify Cardiovascular Risk	244	Oct 2020

NCT: national clinical trial.

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Documentation for Clinical Review

- No records required

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy.

The following codes are included below for informational purposes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy. Policy Statements are intended to provide member coverage information and may include the use of some codes for clarity. The Policy Guidelines section may also provide additional information for how to interpret the Policy Statements and to provide coding guidance in some cases.

Type	Code	Description
CPT®	0119U	Cardiology, ceramides by liquid chromatography-tandem mass spectrometry, plasma, quantitative report with risk score for major cardiovascular events
	0377U	Cardiovascular disease, quantification of advanced serum or plasma lipoprotein profile, by nuclear magnetic resonance (NMR) spectrometry with report of a lipoprotein profile (including 23 variables)
	0401U	Cardiology (coronary heart disease [CHD]), 9 genes (12 variants), targeted variant genotyping, blood, saliva, or buccal swab, algorithm reported as a genetic risk score for a coronary event
	0052U	Lipoprotein, blood, high resolution fractionation and quantitation of lipoproteins, including all five major lipoprotein classes and subclasses of HDL, LDL, and VLDL by vertical auto profile ultracentrifugation
	81291	MTHFR (5,10-methylenetetrahydrofolate reductase) (e.g., hereditary hypercoagulability) gene analysis, common variants (e.g., 677T, 1298C)
	81401	Molecular pathology procedure level 2
	82172	Apolipoprotein, each
	82397	Chemiluminescent assay
	82465	Cholesterol, serum or whole blood, total
	82610	Cystatin C
	82652	Vitamin D; 1, 25 dihydroxy, includes fraction(s), if performed
	82664	Electrophoretic technique, not elsewhere specified
	83090	Homocysteine
	83695	Lipoprotein (a)
	83698	Lipoprotein-associated phospholipase A2 (Lp-PLA2)
	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

Type	Code	Description
	83718	Lipoprotein, direct measurement; high density cholesterol (HDL cholesterol)
	83721	Lipoprotein, direct measurement; LDL cholesterol
	83722	Lipoprotein, direct measurement; small dense LDL cholesterol
	83880	Natriuretic peptide
	84181	Protein; Western Blot, with interpretation and report, blood or other body fluid
	84478	Triglycerides
	85384	Fibrinogen; activity
	85385	Fibrinogen; antigen
	86141	C-reactive protein; high sensitivity (hsCRP)
HCPCS	None	

Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

Effective Date	Action
06/26/2009	BCBSA Medical Policy adoption Existing BSC and adopted BCBSA Policies were combined into a new Policy. The following existing BSC Policies were combined: • Measurement of Small Low-Density Lipoprotein (LDL) Particles and Concentration of LDL Particles in Cardiac Risk Assessment and Management • Lipoprotein(a) Enzyme Immunoassay in the Management of Cardiovascular Disease • High Sensitivity C-Reactive Protein • Homocysteine Testing in the Screening, Diagnosis, and Management of Cardiovascular Disease • Measurement of Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) in the Assessment of Cardiovascular Risk The following BCBSA Medical Policies were adopted and combined: • Apolipoprotein B in the Risk Assessment and Management of Cardiovascular Disease • High-Density Lipoprotein Subclass Testing in the Diagnosis and Management of Cardiovascular Disease • Apolipoprotein E Genotype or Phenotype in the Management of Cardiovascular Disease • Measurement of Serum Intermediate Density Lipoproteins (Remnant-like Particles) • Measurement of Long-Chain Omega-3 Fatty Acids in Red Blood Cell Membranes as a Cardiac Risk Factor The resulting new Policy is Coronary Heart Disease (CHD) - Assessment of Emerging Risk Factors.
11/04/2009	Coding Update
10/12/2012	Policy revision without position change
02/22/2013	Coding Update
11/15/2013	Policy revision with position change
07/31/2015	Coding update

Effective Date	Action
10/30/2015	Policy title change from Coronary Heart Disease (CHD) - Assessment of Emerging Risk Factors Policy revision with position change
12/01/2016	Coding update
02/01/2017	Policy revision without position change
04/01/2018	Policy revision without position change
08/01/2018	Coding update
02/01/2019	Policy revision without position change
03/01/2020	Annual review. No change to policy statement. Literature review updated.
01/01/2021	Coding update
02/01/2021	Annual review. No change to policy statement. Policy guidelines and literature review updated.
03/01/2022	Annual review. Policy statement, guidelines and literature review updated.
02/01/2023	Annual review. No change to policy statement. Policy guidelines and literature review updated.
02/01/2024	Annual review. No change to policy statement. Policy guidelines and literature review updated. Policy title changed from Novel Biomarkers in Risk Assessment and Management of Cardiovascular Disease to current one. Coding update.
04/01/2024	Annual review. Policy statement, guidelines and literature review updated. Updated to combine policies 2.04.32 and 2.04.100 (archived). Coding update.

Definitions of Decision Determinations

Medically Necessary: Services that are Medically Necessary include only those which have been established as safe and effective, are furnished under generally accepted professional standards to treat illness, injury or medical condition, and which, as determined by Blue Shield, are: (a) consistent with Blue Shield medical policy; (b) consistent with the symptoms or diagnosis; (c) not furnished primarily for the convenience of the patient, the attending Physician or other provider; (d) furnished at the most appropriate level which can be provided safely and effectively to the patient; and (e) not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of the Member's illness, injury, or disease.

Investigational/Experimental: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements and Feedback (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

We are interested in receiving feedback relative to developing, adopting, and reviewing criteria for medical policy. Any licensed practitioner who is contracted with Blue Shield of California or Blue Shield of California Promise Health Plan is welcome to provide comments, suggestions, or concerns. Our internal policy committees will receive and take your comments into consideration.

For utilization and medical policy feedback, please send comments to: MedPolicy@blueshieldca.com

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.

Appendix A

POLICY STATEMENT	
BEFORE Red font: Verbiage removed	**AFTER** Blue font: Verbiage Changes/Additions
Biomarker Testing in Risk Assessment and Management of Cardiovascular Disease 2.04.65 **Policy Statement:** I. Measurement of **any** of the following novel lipid and non-lipid biomarkers as an adjunct to low-density lipoprotein cholesterol in the risk assessment and management of cardiovascular disease is considered **investigational.** A. Apolipoprotein AI B. Apolipoprotein B C. Apolipoprotein E D. B-type natriuretic peptide E. Cystatin C F. Fibrinogen G. High-density lipoprotein (HDL) subclass H. Leptin I. Lipoprotein (a) J. Low-density lipoprotein (LDL) subclass	**Biomarker Testing in Risk Assessment and Management of Cardiovascular Disease 2.04.65** **Policy Statement:** **I.** Measurement of **any** of the following nontraditional lipid and non-lipid biomarkers as an adjunct to low-density lipoprotein cholesterol in the risk assessment and management of cardiovascular disease is considered **investigational.** A. Apolipoprotein AI B. Apolipoprotein B C. Apolipoprotein E D. B-type natriuretic peptide E. Cystatin C F. Fibrinogen G. High-density lipoprotein (HDL) subclass **H.** Leptin I. Lipoprotein (a) J. Low-density lipoprotein (LDL) subclass II. Measurement of lipoprotein-associated phospholipase A_2 is considered **investigational**. III. Cardiovascular disease risk panels, consisting of multiple individual biomarkers intended to assess cardiac risk (other than simple lipid panels, see Policy Guidelines section), are considered **investigational**.