The following Protocol contains medical necessity criteria that apply for this service. It is applicable to Medicare Advantage products unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Preauthorization is not required but is recommended if, despite this Protocol position, you feel this service is medically necessary. Please note that payment for covered services is subject to eligibility and the limitations noted in the patient’s contract at the time the services are rendered.

**Description**

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

**Background**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

This Protocol does not address testing performed as a panel.

**Related Protocol**

Genetic Testing for Familial Alzheimer’s Disease

**Policy (Formerly Corporate Medical Guideline)**

Measurement of novel lipid risk factors (i.e., apolipoprotein B, apolipoprotein A-I, apolipoprotein E, B-type natriuretic peptide, cystatin C, fibrinogen, leptin, LDL subclass, HDL subclass, lipoprotein[a]) is considered investigative as an adjunct to LDL cholesterol in the risk assessment and management of cardiovascular disease.

**Medicare Advantage**

For Medicare Advantage this is considered not medically necessary.

Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are considered investigational. For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced procedures. Some of this Protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.

**References**

We are not responsible for the continuing viability of web site addresses that may be listed in any references below.


2. Blue Cross and Blue Shield Association Technology Evaluation Center. C-Reactive Protein as a Cardiac Risk Marker (Special Report). TEC Assessment 2002; Volume 17, Tab 23.


<table>
<thead>
<tr>
<th>Protocol</th>
<th>Novel Biomarkers in Risk Assessment and Management of Cardiovascular Disease</th>
<th>Last Review Date: 03/14</th>
</tr>
</thead>
</table>


73. Superko HR, Berneis KK, Williams PT et al. Gemfibrozil reduces small low-density lipoprotein more in normolipemic subjects classified as low-density lipoprotein pattern B compared with pattern A. Am J Cardiol 2005; 96(9):1266-72.


83. Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial. Am J Cardiol 2002; 90(2):89-94.
84. Otvos JD, Jeyarajah EJ, Cromwell WC. Measurement issues related to lipoprotein heterogeneity. Am J Cardiol 2002; 90(8A):22i-29i.


93. Ridker PM, Hennekens CH, Stampfer MJ. A prospective study of lipoprotein (a) and the risk of myocardial infarction. JAMA 1993; 270(18):2195-9.


