Cardiovascular Disease Risk Tests

Number: 0381

Policy

*Please see amendment for Pennsylvania Medicaid at the end of this CPB.

I. High-sensitivity C-reactive protein (hs-CRP):

A. Aetna considers high-sensitivity C-reactive protein (hs-CRP) testing medically necessary for members who meet all of the following criteria: (i) member has 2 or more coronary heart disease (CHD) major risk factors*, and (ii) member has low-density lipoprotein (LDL) cholesterol levels between 100 to 130 mg/dL; and (iii) member has been judged to be at an intermediate-risk of cardiovascular disease by global risk assessment (i.e., 10 to 20 % risk of CHD per 10 years using Framingham point scoring**).

*Major risk factors include the following:

1. Age (men aged 45 years or older; women aged 55 years or older)
2. Current cigarette smoking
3. Family history of premature CHD (CHD in male first-degree relative less than 55 years of age; CHD in female first-degree relative less than 65 years of age)
4. Hypertension (blood pressure [BP] of 140 mm Hg or higher, or on anti-hypertensive medication)
5. Low high-density lipoprotein (HDL) cholesterol (less than 40 mg/dL).

**Note:** Framingham risk scoring for men and women is presented in the Appendix below.

B. Aetna considers hs-CRP testing experimental and investigative for all other indications, including use as a screening test for the general population and for monitoring response to therapy, because its clinical value for these uses has not been established.

II. Apolipoprotein B (apo B):

Aetna considers measurement of apolipoprotein B (apoB) medically necessary for use in high-risk persons with hypercholesterolemia to assess whether additional intense interventions are necessary when LDL cholesterol goals are reached (LDL cholesterol less than 70 mg/dL and non-HDL cholesterol less than 100 mg/dL in persons with known cardio-vascular disease (CVD) or diabetes mellitus, or LDL-C less than 100 mg/dL and non-HDL cholesterol less than 130 mg/dL in persons with other risk factors). High-risk persons are those with 1 or more of the following criteria:

A. Diabetes mellitus; or
B. Known CVD; or
C. Two or more of the following CVD risk factors:
   1. Current cigarette smoking; or
   2. Family history of premature CVD (CHD in male first-degree relative less than 55 years of age; CHD in female first-degree relative less than 65 years of age); or
   3. Hypertension (BP of 140 mm Hg or higher, or on anti-hypertensive medication).

Aetna considers measurement of apolipoprotein B (apoB)
experimental and investigational for all other indications because its clinical value for other indications has not been established.

III. Aetna considers any of the following tests for assessing CHD risk experimental and investigational because their clinical value has not been established:

A. Activated factor VII
B. Adiponectin
C. Angiotensin gene (CardiaRisk AGT)
D. Anti-thrombin III
E. Apelin
F. Apolipoprotein A-I (apo Al) (Boston Heart HDL Map panel)
G. Apolipoprotein E (apo E)
H. Apolipoproteins E genotyping
I. ASCVD risk testing (individual or panel) (eg, c-peptide, islet cell antibodies, nonesterified fatty acids (free fatty acids), proinsulin and total insulin)
J. B-type natriuretic peptides (see CPB 0618 - Brain Natriuretic Peptide Testing (../600_699/0618.html))
K. Carotid ultrasound screening of asymptomatic persons for carotid artery stenosis
L. Chromosome 9 polymorphism 9p21
M. Circulating microRNAs (e.g., miR-1, miR-16, miR-26a, miR-27a, and miR-29a, miR-133a, and miR-199a-5p; not an all-inclusive list)
N. Coenzyme Q10 (CoQ10)
O. Coronary artery reactivity test
P. Cystatin-C
Q. Factor II (thrombin) (F2 gene)
R. Factor V Leiden (F5 gene)
S. Fibrinogen
T. 4q25 genotype testing (eg, 4q25-AF Risk Genotype Test, Cardio IQ 4q25-AF Risk Genotype Test)
U. Galectin-3
V. Genetic testing (for genetic testing for familial hypercholesterolemia, see CPB 140 - Genetic Testing)
W. Growth stimulation expressed gene 2 (ST2)
X. HDL subspecies (LpAl, LpAl/AII and/or HDL3 and HDL2)
Y. Homocysteine testing
Z. Interleukin 6 (IL-6)
AA. Interleukin 6 -174 g/c promoter polymorphism
AB. Interleukin 17 gene polymorphism
AC. Kinesin-like protein 6 (KLP6)
AD. LDL gradient gel electrophoresis
AE. LDL subspecies (small and large LDL particles)
AF. Leptin
AG. Lipidomic and metabolomic risk markers
AH. Lipoprotein remnants: intermediate density lipoproteins (IDL) and small density lipoproteins
AI. Lipoprotein(a) (Lp(a)) enzyme immunoassay
AJ. Lipoprotein-associated phospholipase A2 (Lp-PLA2) (PLAC)
AK. Long chain omega-3 fatty acids composition in red blood cell
AL. LPA Intron-25 genotype testing (eg, Cardio IQ Intron-25 Genotype Test, LPA Intron-25 Genotype Test)
AM. MaxPulse testing
AN. Measurement of free fatty acids
AO. Mid-regional pro-atrial natriuretic peptide
AP. MIRISK VP test
AQ. Myeloperoxidase (MPO)
AR. NMR Lipoprofile
AS. Osteoprotegerin
AT. Oxidized phospholipids
AU. Peroxisome proliferator-activated receptor
AV. Plasminogen activator inhibitor (PAI–1)
AW. Pregnancy-associated plasma protein-A (PAPP-A)
AX. Protein C
AY. Prothrombin gene mutation testing
AZ. Resistin
BA. Retinol binding protein 4 (RBP4)
BB. Serum sterols (eg, Boston Heart Cholesterol Balance Test)
BC. Singulex SMC testing for risk of cardiac dysfunction and vascular inflammation (eg, SMC Endothelin, SMC IL-6, SMC IL 17A, SMC c TnI and SMC TNF-α)
BD. Skin cholesterol (eg, PREVU)
BE. SNP-based testing (eg, Cardiac Healthy Weight DNA Insight, Healthy Woman DNA Insight Test, Heart Health Genetic Test)
BF. Thromboxane metabolite(s) testing
BG. Tissue plasminogen activator (tPA)
BH. Toll-like receptor 4 (TLR4) Asp299Gly (rs4986790) polymorphism
BI. Troponin I (eg, PATHFAST cTnI-II)
BJ. Tumor necrosis factor alpha (TNF-α)
BK. Total cholesterol content in red blood cell membranes
BL. Vertical Auto Profile (VAP) with or without vertical lipoprotein particle (VLP) technology
BM. Visfatin
BN. von Willebrand factor antigen level.

The medical literature does not support the utility of the above tests for screening, diagnosis, or management of CHD.

IV. Aetna considers homocysteine testing experimental and investigational for assessing CHD or stroke risk and for evaluating women with recurrent pregnancy loss (see CPB 348 - Recurrent Pregnancy Loss (http://aetnet.aetna.com/mpa/cpb/300_399/0347.html)). Homocysteine testing may be medically necessary for the following indications: (i) evaluating persons with homocystinuria (cystathionine beta synthase deficiency); (ii) evaluating persons with coagulation disorders (e.g., unexplained thrombotic disorders such as deep venous thrombosis or pulmonary embolism); and (iv) for evaluating persons with borderline vitamin B12 deficiency (see CPB 0536 - Vitamin B-12 Therapy (../500_599/0536.html)). Homocysteine testing is considered experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established (see CPB 0763 - Homocysteine Testing (../700_799/0763.html)).

V. Aetna considers measurement of carotid intima-media
thickness experimental and investigational for assessing CHD risk because its effectiveness has not been established.

VI. Aetna considers noninvasive measurements of arterial elasticity by means of blood pressure waveforms (e.g., CardioVision MS-2000, CVProfilor, Digital Pulse Analyzer (DPA), Max Pulse and HDI PulseWave) and noninvasive calculation and analysis of central arterial pressure waveforms (SphygmoCor) experimental and investigational for assessing CHD risk because their effectiveness has not been established.

VII. Aetna considers peripheral arterial tonometry (e.g., the Endo-PAT2000/EndoPAT device) experimental and investigational for assessing CHD because there is insufficient evidence to support the effectiveness of this approach.

VIII. Aetna considers the Corus CAD gene expression profile medically necessary for evaluation of nondiabetic adults with chest pain or anginal equivalent symptoms who have no history of obstructive coronary artery disease. The Corus CAD is considered experimental and investigational for persons with a history of myocardial infarction, current MI or acute coronary syndrome, current New York Heart Association (NYHA) class III or IV congestive heart failure symptoms, any previous coronary revascularization, persons with suspected unstable angina, persons with systemic infections, persons with systemic inflammatory conditions, and persons currently taking steroids, immunosuppressive agents, or chemotherapeutic agents. The Corus CAD is considered experimental and investigational for all other indications.

IX. Aetna considers stress echocardiography experimental and investigational for cardiovascular disease risk assessment in asymptomatic low risk individuals.

X. Aetna considers ultrasound of the upper and
lower extremity arteries experimental and investigational for screening of persons without signs or symptoms of peripheral arterial disease.

XI. Aetna considers venous ultrasound experimental and investigational for screening of persons without signs or symptoms of peripheral venous disease. and who are not at high risk for venous thromboembolic disorders.

For coverage criteria for PCSK9 inhibitors (alirocumab (Praluent)), see Pharmacy Clinical Policy Bulletin (PCPB) - PCSK9 Inhibitors.

See also CPB 0228 - Cardiac CT, Coronary CT Angiography and Calcium Scoring (../200_299/0228.html); CPB 0525 - Screening for Lipid Disorders (../500_599/0525.html).

Background
Cardiovascular disease (CVD) risk testing is utilized to indicate the chances of having a coronary event. The most common tests to determine cardiac risk are high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol and triglycerides (often referred to as a basic or standard lipid panel).

Non-traditional risk factors for coronary heart disease (CHD) are used increasingly to determine patient risk, in part because of an assumption that many patients with CHD lack traditional risk factors (e.g., cigarette smoking, diabetes, hyperlipidemia, and hypertension).

Hackman and Anand (2003) summarized existing evidence about the connection between atherosclerotic vascular disease and certain nontraditional CHD risk factors (abnormal levels of C-reactive protein [CRP], fibrinogen, lipoprotein(a), and homocysteine [Hcy]). The authors conclude that current evidence does not support the notion that non-traditional risk assessment adds overall value to traditional risk assessment. The authors explained that “for each putative risk factor, there
must be prospective controlled trials demonstrating that (i) targeting individuals with elevated levels of these risk factors for proven risk-reducing interventions offers advantages over current methods of targeting therapy (e.g., by cholesterol, diabetes, and blood pressure screening); or (ii) selectively and specifically reducing the risk factor reduces hard cardiovascular end points, such as mortality, nonfatal myocardial infarction, and stroke.”

Large prospective studies support screening for traditional risk factors. In one study, Greenland et al (2003) assessed major antecedent risk factors among patients who suffered fatal CHD or non-fatal myocardial infarction (MI) while enrolled in 3 prospective cohort studies involving nearly 400,000 patients (age range of 18 to 59). Follow-up ranged from 21 to 30 years. Major risk factors were defined as total cholesterol greater than or equal to 240 mg/dL (greater than or equal to 6.22 mmol/L), systolic blood pressure (BP) greater than or equal to 140 mm Hg, diastolic BP greater than or equal to 90 mm Hg, current cigarette smoking, and diabetes. Of patients age 40 to 59 at baseline who died of CHD during the 3 studies, 90 % to 94 % of women and 87 % to 93 % of men had at least 1 major CHD risk factor. In the 1 study that assessed non-fatal MI, at least 1 major risk factor was present in 87 % of women and 92 % of men age 40 to 59.

In another large study (Khot et al, 2003), researchers analyzed data from more than 120,000 patients enrolled in 14 randomized controlled trials (RCTs) to determine the prevalence of baseline conventional risk factors among CHD patients. Of patients with CHD, 85 % of women and 81 % of men had at least 1 conventional risk factor.

As Canto and Iskandrian (2003) notes, these data challenge the assumption that “only 50 %” of CHD is attributable to conventional risk factors and emphasize the importance of screening for these risk factors and aggressively treating patients who have them.
An assessment by the BlueCross BlueShield Association Technology Evaluation Center (BCBSA, 2005) provided a framework for the evaluation of the potential clinical utility of putative risk factors for cardiovascular disease. The assessment explained that the strongest evidence of the value of such a test is direct evidence that its measurement to assess cardiovascular disease risk results in improved patient outcomes. In the absence of such evidence, the assessment of the potential clinical utility of a test lies in understanding a chain of logic and the evidence supporting those links in the chain. The potential for clinical utility of a test for assessing cardiovascular disease risk lies in following a chain of logic that relies on evidence regarding the ability of a measurement to predict cardiovascular disease beyond that of current risk prediction methods or models, and evidence regarding the utility of risk prediction to treatment of cardiovascular disease. In order to assess the utility of a test in risk prediction, specific recommendations regarding patient management based on the test results should be stated. The assessment notes that another factor that would be important to consider is the availability and reliability of laboratory measurements.

In a report on the use of non-traditional risk factors in CHD risk assessment, the U.S. Preventive Services Task Force (USPSTF, 2009) stated that there is insufficient evidence to recommend the use of non-traditional risk factors to screen asymptomatic individuals with no history of CHD to prevent CHD events. Treatment to prevent CHD events by modifying risk factors is currently based on the Framingham risk model. Risk factors not currently part of the Framingham model (i.e., non-traditional risk factors) include high sensitivity CRP (hs-CRP), ankle-brachial index (ABI), leukocyte count, fasting blood glucose level, periodontal disease, carotid intima-media thickness, electron beam computed tomography, Hcy level, and lipoprotein(a) level.

To determine if non-traditional risk factors could play a role in determining those at high-risk for CHD, the USPSTF reviewed the published literature and found the availability and validity
of the evidence varied considerably (USPSTF, 2009). They said there is insufficient evidence to determine the percentage of intermediate-risk individuals who would be re-classified by screening with non-traditional risk factors, other than hs-CRP and ABI. For individuals re-classified as high-risk on the basis of hs-CRP or ABI scores, data are not available to determine whether they benefit from additional treatments. In addition, there is not enough information available about the benefits and harms of using non-traditional risk factors in screening. Potential harms include lifelong use of medications without proven benefit and psychological and other harms from being mis-classified in a higher risk category. The USPSTF stated that clinicians should continue to use the Framingham model to assess CHD risk and guide risk-based preventive therapy (USPSTF, 2009).

**High Sensitivity C-Reactive Protein (hs-CRP):**

C-reactive protein (CRP) is produced by the liver. An elevated CRP level may be indicative of inflammation (nonspecific location). hs-CRP can detect the slight elevations in serum CRP that are associated with coronary artery disease (CAD), which can be within the normal range.

It has been theorized that certain markers of inflammation -- both systemic and local -- may play a role in the development of atherosclerosis. High sensitivity CRP (hs-CRP) is one systemic marker of inflammation that has been intensively studied and identified as an independent risk factor for coronary artery disease (CAD). Of current inflammatory markers identified, hs-CRP has the analyte and assay characteristics most conducive for use in practice. A Writing Group convened by the American Heart Association and the Centers for Disease Control and Prevention (Pearson et al, 2003) endorsed the optional use of hs-CRP to identify persons without known cardiovascular disease who are at intermediate risk (10 to 20 % risk of coronary heart disease over the next 10 years). For these patients, the results of hs-CRP testing may help guide considerations of further evaluation (e.g., imaging, exercise
testing) or therapy (e.g., drug therapies with lipid-lowering, anti-platelet, or cardio-protective agents). The Writing Group noted, however, that the benefits of such therapy based on this strategy remain uncertain. High-sensitivity CRP testing is not necessary in high-risk patients who have a 10-year risk of greater than 20%, as these patients already qualify for intensive medical interventions. Individuals at low-risk (less than 10% per 10 years) will be unlikely to have a high-risk (greater than 20%) identified through hs-CRP testing. The Writing group recommended screening average risk (10-year risk less than 10%) for hs-CRP for purposes of cardiovascular risk assessment. The Writing Group stated that hs-CRP also may be useful in estimating prognosis in patients who need secondary preventive care, such as those with stable coronary disease or acute coronary syndromes and those who have undergone percutaneous coronary interventions. The Writing Group posited that this information may be useful in patient counseling because it offers motivation to comply with proven secondary preventive interventions. However, the Writing Group noted that the utility of hs-CRP in secondary prevention is more limited because current guidelines for secondary prevention generally recommend, without measuring hs-CRP, the aggressive application of secondary preventive interventions. The Writing Group recommends measurement of hs-CRP be performed twice (averaging results), optimally 2 weeks apart, fasting or non-fasting in metabolically stable patients. Patients with an average hs-CRP level greater than 3.0 mg/dL are considered to be at high relative risk of CHD. Patients with an average hs-CRP level less than 1 mg/L are at low relative risk, and patients with an hs-CRP level between 1.0 and 3.0 mg/L are at average relative risk. If hs-CRP level is greater than 10 mg/dL, the Writing Group recommends that testing should be repeated and the patient examined for sources of infections or inflammation. The Writing group recommended against the measurement of inflammatory markers other than hs-CRP (cytokines, other acute-phase reactants) for determination of coronary risk in addition to hs-CRP.
In an analysis of Women's Health Study participants, including hs-CRP in cardiovascular disease (CVD)-risk prediction improved the predictive accuracy in non-diabetic women whose traditional 10-year CVD risk was at least 5%. Cook et al (2006) compared risk-prediction models that include or do not include hs-CRP. The models were applied to 15,048 Women's Health Study participants who were age 45 or older and free of cardiovascular disease and cancer at baseline. During a mean follow-up of 10 years, 390 women developed CVD. For accurately predicting CVD events, hs-CRP was out-matched only by older age, current smoking, and high blood pressure among traditional Framingham variables. Non-diabetic women were classified according to their 10-year risk for CVD in a model without CRP. Adding CRP to the model substantially improved predictive accuracy for women with an initial 10-year CVD risk of at least 5%. The gain in accuracy was greatest among women initially classified in the 5% to 9.9% risk range: 21.3% of those women were re-classified in a more accurate risk category when CRP was included in the risk-prediction model (11.9% moved down a risk category (to less than 5%) and 9.5% moved up a risk category (to 10% to 19.9%)). Accounting for the predictive value of older age, smoking, and high BP lessened the predictive contribution of CRP but still left CRP ahead of any cholesterol parameter (total, LDL, or HDL).

In a nested, case-control study of 122 cases and 244 controls drawn from a cohort of Women's Health Study participants, Ridker et al (2000) assessed the risk for CVD according to levels of 4 inflammatory markers: hs-CRP, serum amyloid A, interleukin-6, and soluble intercellular adhesion molecule type-1 (sICAM-1). Homocysteine and several lipid and lipoprotein fractions (including apolipoprotein A-I, apolipoprotein B-100, lipoprotein(a), total cholesterol and HDL cholesterol) were measured. Outcomes included fatal CHD, non-fatal MI, stroke, or coronary re-vascularization procedures. Overall, hs-CRP showed the strongest univariate association with all markers studied. Although several other markers studies were univariate predictors of CVD, hs-CRP was the only novel plasma marker that predicted risk in multi-variate
analysis. Total cholesterol-to-HDL ratio also predicted risk in multi-variate analysis.

Yeh (2005) noted that as a clinical tool for assessment of cardiovascular risk, hs-CRP testing enhances information provided by lipid screening or global risk assessment. While statin therapy and other interventions can reduce hs-CRP, whether or not such reductions can actually prevent cardiovascular events is being investigated. This is in agreement with the observation of Nambi and Ballantyne (2005) who stated that studies are now under way to evaluate if targeting patients with high CRP and low LDL cholesterol will have any impact on future cardiovascular events and survival and whether changes in CRP correlate to event reduction.

Evidence from the JUPITER trial suggests that, for people choosing to start statin therapy, reduction in both LDL cholesterol and hsCRP are indicators of successful treatment with statins (Ridker et al, 2009). In an analysis of 15,548 initially healthy men and women participating in the JUPITER trial (87 % of full cohort), investigators prospectively assessed the effects of rosuvastatin versus placebo on rates of non-fatal myocardial infarction, non-fatal stroke, admission for unstable angina, arterial re-vascularisation, or cardiovascular death during a maximum follow-up of 5 years (median of 1.9 years). Compared with placebo, participants allocated to rosuvastatin who achieved LDL cholesterol less than 1.8 mmol/L had a 55 % reduction in vascular events, and those achieving hsCRP less than 2 mg/L a 62 % reduction. Although LDL cholesterol and hs-CRP reductions were only weakly correlated in individual patients (r values < 0.15), the investigators reported a 65 % reduction in vascular events in participants allocated to rosuvastatin who achieved both LDL cholesterol less than 1.8 mmol/L and hs-CRP less than 2 mg/L, versus a 33 % reduction in those who achieved 1 or neither target. In participants who achieved LDL cholesterol less than 1.8 mmol/L and hs-CRP less than 1 mg/L, the investigators found a 79 % reduction. The investigators reported that achieved hs-CRP concentrations were predictive of event rates irrespective of the lipid endpoint
used, including the apolipoprotein B to apolipoprotein AI ratio (Ridker et al, 2009).

A meta-analysis found that hsCRP concentration has continuous associations with the risk of coronary heart disease, ischemic stroke, and vascular mortality (Emerging Risk Factors Collaboration, 2010). Investigators assessed the associations of hs-CRP concentration with risk of vascular and non-vascular outcomes under different circumstances. Investigators meta-analyzed individual records of 160,309 people without a history of vascular disease (i.e., 1.31 million person-years at risk, 27,769 fatal or non-fatal disease outcomes) from 54 long-term prospective studies. Within-study regression analyses were adjusted for within-person variation in risk factor levels. The investigators found that log(e) hs-CRP concentration was linearly associated with several conventional risk factors and inflammatory markers, and nearly log-linearly with the risk of ischemic vascular disease and non-vascular mortality. Risk ratios (RRs) for coronary heart disease per 1 standard deviation higher log(e) hs-CRP concentration (3-fold higher) were 1.63 (95% confidence interval (CI): 1.51 to 1.76) when initially adjusted for age and sex only, and 1.37 (1.27 to 1.48) when adjusted further for conventional risk factors; 1.44 (1.32 to 1.57) and 1.27 (1.15 to 1.40) for ischemic stroke; 1.71 (1.53 to 1.91) and 1.55 (1.37 to 1.76) for vascular mortality; and 1.55 (1.41 to 1.69) and 1.54 (1.40 to 1.68) for non-vascular mortality. The investigators noted that RRs were largely unchanged after exclusion of smokers or initial follow-up. After further adjustment for fibrinogen, the corresponding RRs were 1.23 (1.07 to 1.42) for coronary heart disease; 1.32 (1.18 to 1.49) for ischemic stroke; 1.34 (1.18 to 1.52) for vascular mortality; and 1.34 (1.20 to 1.50) for non-vascular mortality. The investigators concluded that hs-CRP concentration has continuous associations with the risk of coronary heart disease, ischemic stroke, vascular mortality, and death from several cancers and lung disease that are each of broadly similar size. The investigators noted that the relevance of hs-CRP to such a range of disorders is unclear. The investigators found that associations with ischemic vascular disease depend
considerably on conventional risk factors and other markers of inflammation.

According to guidelines from the National Academy of Clinical Biochemistry (2009), if global risk is intermediate and uncertainty remains as to the use of preventive therapies, hs-CRP measurement might be useful for further stratification into a higher or lower risk category. Guidelines from the American College of Cardiology/American Heart Association (2010) also address the selection of patients for statin therapy, stating it can be useful in men 50 years or older and women 60 years of age or older with LDL-C less than 130 mg/dL; not on lipid-lowering, hormone replacement, or immunosuppressant therapy; without clinical coronary heart disease, diabetes, chronic kidney disease, severe inflammatory conditions, or contraindications to statins.

Guidelines from the Canadian Cardiovascular Society (2009, 2013) state that the measurement of hs-CRP is being recommended in men older than 50 years and women older than 60 years of age who are at intermediate risk (10% to 19%) according to their Framingham risk score and who do not otherwise qualify for lipid-lowering therapy (i.e., if their LDL-C is less than 3.5 mmol/L). The guidelines explain that the rationale for measuring hs-CRP specifically in these individuals is that we now have class I evidence for the benefit of statin therapy in such individuals, if their hs-CRP is greater than 2.0 mg/L. The guidelines found that data from the JUPITER study show that statin therapy reduces cardiovascular events (hazard ratio 0.56 [95% CI 0.46 to 0.69]; P<0.00001). The guidelines note, because hs-CRP can be elevated during acute illness, clinical judgment should be exercised in the interpretation of any single measurement of hs-CRP. Canadian Cardiovascular Society guidelines (2013) state that those subjects who meet JUPITER criteria (men greater than 50 years and women greater than 60 years of age and CRP greater than or equal to 2 mg/L and LDL greater than 3.5 mmol/L) could be considered for treatment based on the results of that study.
An American Heart Association statement on nontraditional risk factors and biomarkers in cardiovascular disease in youth (Balagopal, et al., 2011) stated: "There currently is no clinical role for measuring CRP routinely in children when assessing or considering therapy for CVD risk factors." The AHA statement explains that, although numerous studies suggest that CRP is elevated in children with higher CVD risk, correlates with the progression of atherosclerotic changes, and tracks, albeit weakly, over 21 years from childhood to adulthood independently of other metabolic and conventional cardiovascular risk factors, it is not yet clear whether high CRP levels during childhood and adolescence lead to an increased risk of CVD in later life. The AHA stated that lifestyle interventions have been shown to decrease CRP in children, and statins reduce CRP in adults. "However, minimal information is available on the effect of statins on CRP in children and youth and, importantly whether lowering CRP in children per se would modify preclinical disease or CVD outcomes."

An assessment prepared for the Agency for Healthcare Research and Quality (Helfand, et al., 2009) found that, "across all of the criteria listed in the table, C-reactive and electron beam computed tomography scan had the strongest evidence for an independent effect in intermediate-risk individuals, and both reclassify some individuals as high-risk."

An National Heart Lung and Blood Institute (2012) guideline on cardiovascular disease risk in children and adolescents found insufficient evidence to recommend the measurement of inflammatory markers in youths.

The American Association of Clinical Endocrinologists (2012) have a 2b recommendation for the use of hs-CRP to stratify CVD risk in patients with a standard risk assessment that is borderline, or in those with an LDL-C concentration less than 130 mg/dL.

A European consensus guideline (2012) included a strong
recommendation that hs-CRP should not be measured in asymptomatic low-risk individuals and high-risk patients to assess 10-year risk of CVD. The guideline included a weak recommendation that high-sensitivity CRP may be measured as part of refined risk assessment in patients with an unusual or moderate CVD risk profile.

Lipoprotein (a) Enzyme Immunoassay

Lipoprotein(a) testing (Lp[a]) is an LDL cholesterol particle that is attached to a special protein called apo A. Elevated levels in the blood are purportedly linked to a greater likelihood of atherosclerosis and heart attacks.

The lipoprotein(a) (Lp(a)) enzyme immunoassay have been promoted as an important determinant of CHD risk, and as a guide to drug and diet therapy in patients with established CAD.

Although there is evidence for an association of Lp(a) with cardiovascular disease, there are no data to suggest that more aggressive risk factor modification would improve patient-oriented health outcomes (Pejic and Jamieson, 2007). Furthermore, it is very difficult to modify Lp(a). Some studies suggest that it can be lowered using high doses of niacin, neomycin, or estrogen in women (e.g., Gurakar et al, 1985).

Braunwald et al states “because Lp(a) measurement is not a widely available laboratory determination and the clinical significance of alterations in Lp(a) is not known, the NCEP [National Cholesterol Education Program] does not recommend the routine measurement of this lipoprotein at this time.”

Prospective studies that evaluated Lp(a) as a predictor of cardiovascular events have had conflicting results. Some studies suggested that Lp(a) was an independent risk factor for CHD (Bostom et al, 1994; Bostom et al, 1996; Schaefer et al, 1994; Nguyen et al, 1997; Wald et al, 1994; Cremer et al, 1994; Schwartzman et al, 1998; Ariyo et al, 2003; Shai et al, 2003), while others showed no significant association (Coleman et al,
1992; Ridker et al, 1993; Jauhiainen et al, 1991; Cantin et al, 1998; Nishino et al, 2000). A meta-analysis of 5,436 patients followed for at least 1 year concluded that elevated Lp(a) is associated with increased cardiovascular risk (relative risk 1.6; 95 % CI: 1.4 to 1.8) (Danesh et al, 2000).

Hackam and Anand (2003) systematically reviewed the evidence for Lp(a) and concluded that “the use of Lp(a) as a screening tool has some limitations.” Although they identified moderate evidence for its role as an independent risk factor, they found minimal information on its incremental risk, and no prospective clinical outcome studies evaluating its role in management.

Although some studies have linked elevated serum levels of Lp(a) to cardiovascular risk, the clinical utility of this marker has not been established. Suk Danik et al (2006) analyzed data available from a cohort of about 28,000 participants followed for 10 years in the Women’s Health Study. Blood samples that had been frozen at study entry were tested for lipoprotein(a), and incident cardiovascular events were documented during the follow-up period. A total of 26 % of the women had lipoprotein(a) levels greater than 30 mg/dL, which is the level currently considered to confer increased cardiovascular risk. However, only the women in the highest quintile with respect to lipoprotein(a) level (greater than or equal to 44 mg/dL) were more likely to experience cardiovascular events than women in the lowest quintile (hazard ratio [HR], 1.47); thus, a threshold effect was seen. Overall, women with the highest rates of cardiovascular disease were those who had lipoprotein(a) levels at or above the 90th percentile and LDL-C levels at or above the median. These findings indicate that routinely measuring lipoprotein(a) is of little benefit for most women. However, lipoprotein(a) testing might be helpful in the clinical management of women who are at particularly high-risk or who have already experienced a cardiovascular event despite having few or no traditional risk factors. Since lipoprotein(a) is not decreased by lipid-lowering therapies, the mainstay of therapy for cardiovascular risk is still aggressive control of LDL-C levels.
with a statin or niacin, regardless of a woman’s lipoprotein(a) level.

A study by Ariyo et al (2003) of the predictive value of Lp(a) in the elderly (age greater than 65 years) found that lipoprotein(a) levels have prognostic value for stroke and death in men, but not for CHD in men or for any major vascular outcome in women. However, even the links for stroke and death in men were evident only in the highest compared with the lowest quintile, not in intermediate quintiles. Ariyo et al (2003) prospectively studied 3,972 Cardiovascular Health Study participants (minimum age of 65) who had Lp(a) measurements taken at baseline and did not have vascular disease. Overall, mean baseline Lp(a) levels were slightly higher among women (4.4 mg/dL) than among men (3.9 mg/dL). Median follow-up was 7.4 years. Study participants were placed into quintiles of Lp(a) level (lowest, 0.1 to 1.2 mg/dL; highest, 8.2 to 47.5 mg/dL). In analyses adjusted for other vascular-disease risk factors, elderly women in the highest Lp(a) quintile were no more likely to experience stroke, CHD, death from vascular causes, or death from any cause than were elderly women in the lowest quintile. However, compared with elderly men in the lowest Lp(a) quintile, elderly men in the highest quintile were significantly more likely to experience stroke (HR, 2.92), death from vascular causes (HR, 2.09), and death from any cause (HR, 1.60), but not CHD. The authors concluded that, overall, these results do not appear to support routine measurement of Lp(a) levels in elderly persons.

A meta-analysis found independent but modest associations of Lp(a) concentration with risk of CHD and stroke (Emerging Risk Factors Collaboration, 2009). To assess the relationship of Lp(a) concentration with risk of major vascular and non-vascular outcomes, the investigators examined long-term prospective studies that recorded Lp(a) concentration and subsequent major vascular morbidity and/or cause-specific mortality published between January 1970 and March 2009. Individual records were provided for each of 126,634 participants in 36 prospective studies. During 1.3 million person-years of
follow-up, 22,076 first-ever fatal or non-fatal vascular disease outcomes or non-vascular deaths were recorded, including 9,336 CHD outcomes, 1,903 ischemic strokes, 338 hemorrhagic strokes, 751 unclassified strokes, 1,091 other vascular deaths, 8,114 nonvascular deaths, and 242 deaths of unknown cause. Within-study regression analyses were adjusted for within-person variation and combined using meta-analysis. Analyses excluded participants with known pre-existing CHD or stroke at baseline. The investigators reported that Lp(a) concentration was weakly correlated with several conventional vascular risk factors and it was highly consistent within individuals over several years. The investigators also found that associations of Lp(a) with CHD risk were broadly continuous in shape. In the 24 cohort studies, the rates of CHD in the top and bottom thirds of baseline Lp(a) distributions, respectively, were 5.6 (95 % CI: 5.4 to 5.9) per 1,000 person-years and 4.4 (95 % CI: 4.2 to 4.6) per 1,000 person-years. The risk ratio for CHD, adjusted for age and sex only, was 1.16 (95 % CI: 1.11 to 1.22) per 3.5-fold higher usual Lp(a) concentration (i.e., per 1 standard deviation), and it was 1.13 (95 % CI: 1.09 to 1.18) following further adjustment for lipids and other conventional risk factors. The corresponding adjusted risk ratios were 1.10 (95 % CI: 1.02 to 1.18) for ischemic stroke, 1.01 (95 % CI: 0.98 to 1.05) for the aggregate of non-vascular mortality, 1.00 (95 % CI: 0.97 to 1.04) for cancer deaths, and 1.00 (95 % CI: 0.95 to 1.06) for non-vascular deaths other than cancer.

A genetic association study identified 2 single nucleotide polymorphisms that were strongly associated with both an increased level of Lp(a) lipoprotein and an increased risk for coronary artery disease, providing support for a causal role of Lp(a) lipoprotein in CAD (Clarke et al, 2009). Investigators assessed 2,100 candidate genes in 3,145 case patients with CAD and 3,352 controls. Single-nucleotide polymorphisms (SNPs) mapped to 3 chromosomal regions (6q26-27, 9p21, and 1p13) associated with Lp(a) lipoprotein were significantly associated with CAD risk. An accompanying editorial (Katherisan, 2009) stated: "Although the appropriate role of plasma Lp(a) lipoprotein in risk assessment remains a subject of debate,
there is likely to be increased enthusiasm for measuring plasma Lp(a) lipoprotein levels (and possibly LPA genetic variants) to assess the risk of coronary disease. Additional studies are needed to determine whether knowledge regarding Lp(a) lipoprotein will prove to be clinically useful with respect to risk discrimination, calibration, or reclassification." In particular, the editorialist stated: "To close the loop for plasma Lp(a) lipoprotein from a curiosity to a causal risk factor, a therapeutic intervention that selectively lowers the plasma Lp(a) lipoprotein level will need to be tested in a randomized clinical trial" (Katherisan, 2009).

In a nested case-control study, lipoprotein(a) was found to add little to standard lipid measures and CRP in predicting development of peripheral arterial disease. Ridker et al (2001) had access to baseline plasma samples from 14,916 healthy men from the Physicians' Health Study. Samples from 140 cases who developed symptomatic peripheral arterial disease (PAD) during 9-year follow-up were compared with samples from 140 controls (matched by age, smoking status, and length of follow-up) who did not develop PAD. Eleven standard and novel biomarkers were analyzed. Most biomarkers were significant independent predictors of PAD. Ratio of total cholesterol (TC) to HDL cholesterol was the strongest lipid predictor (adjusted relative risk, 3.9; 95% CI: 1.7 to 8.6); CRP was the strongest non-lipid predictor (adjusted RR, 2.8; 95% CI: 1.3 to 5.9). In a separate analysis of which novel biomarkers would enhance the predictive power of standard lipid measures (TC and TC/HDL ratio), the inflammatory markers (fibrinogen and CRP) were the only ones to add to it significantly (CRP even more than fibrinogen). As expected, lipoprotein(a) and Hcy added little, as did LDL cholesterol, apolipoprotein A-1, and apolipoprotein B-100.

No universally accepted, standardized method for determination for Lp(a) exists, although a working group of the International Federation of Clinical Chemistry demonstrated the inaccuracy of Lp(a) values determined by methods sensitive to apo(a) size and recommended the widespread implementation
of a proposed reference material for those Lp(a) assays that are validated to be unaffected by apo(a) size heterogeneity (Tate et al, 1998; Tate et al, 1999; Marcovina et al, 2000). Lipoprotein(a) concentrations are unaffected by most available lipid-lowering therapies, with the exception of high-dose nicotinic acid, which is often poorly tolerated. This has made it difficult to demonstrate that Lp(a) plays a direct role in vascular disease, since large-scale controlled intervention studies examining the reduction of Lp(a) and hard cardiovascular end points have not been performed. Lastly, the incremental predictive value of Lp(a) measurement additive to that of traditional screening methods for global risk assessment has not been formally studied.

There is no uniform guideline recommendation for the use of Lp(a) in assessment of cardiovascular disease risk. The U.S. Preventive Services Task Force (USPSTF, 2009) does not recommend the use of Lp(a) for cardiovascular screening. The USPSTF (2009) concluded that there is insufficient evidence to recommend the use of lipoprotein(a) level to screen asymptomatic individuals with no history of CHD to prevent CHD events.

An assessment prepared for the Agency for Healthcare Quality and Research (Helfand, et al., 2009) concluded that "lipoprotein(a) probably provides independent information about coronary heart disease risk, but data about their prevalence and impact when added to Framingham risk score in intermediate-risk individuals are limited."

An assessment by the National Academy of Clinical Biochemistry (Cooper et al, 2009) stated that lipoprotein (a) screening is not warranted for primary prevention and assessment of cardiovascular risk. However, if risk is intermediate (10 % to 20 %) and uncertainty remains as to the use of preventive therapies such as statins or aspirin, then lipoprotein (a) measurement "may be done at the physician’s discretion." The assessment also stated that, after global risk assessment, lipoprotein (a) measurements in patients with a
strong family history of premature CVD "may be useful" for identifying individuals having a genetic predisposition of CVD. The assessment stated, however, that benefits of therapies based on lipoprotein (a) concentrations are uncertain. If both lipoprotein (a) and LDL-C are highly increased, "an attempt can be made at the physician’s discretion to lower lipoprotein (a) level by lowering the elevated LDL-C." The assessment stated that there is insufficient evidence to support therapeutic monitoring of lipoprotein (a) levels for evaluating the effects of treatment. The assessment also stated that population routine testing for small size apolipoprotein (a) is not warranted.

A consensus statement by the American College of Cardiology (ACC) and the American Diabetes Association (ADA) (Brunzell et al, 2008) concluded that the clinical utility of routine measurement of Lp(a) is unclear, although more aggressive control of other lipoprotein parameters may be warranted in those with high concentrations of Lp(a).

A European consensus statement (2012) found that high concentrations of Lp(a) are associated with increased risk of CHD and ischemic stroke, although there is no randomized intervention showing that reducing Lp(a) decreases CVD risk. The guidelines concluded that there is no justification for screening the general population for Lp(a) at present, and no evidence that any value should be considered as a target.

Canadian Cardiovascular Society guidelines (2013) state that measurement of Lp(a) might be of value in additional risk assessment particularly in individuals with a family history of premature vascular disease and familial hypercholesterolemia. The guidelines, however, make no recommendation for use of Lp(a) in cardioavascular disease risk assessment.

Guidelines from the American Academy of Clinical Endocrinology (2012) state that testing for lipoprotein (a) is not generally recommended, although it may provide useful information to ascribe risk in white patients with CAD or in those with an unexplained family history of early CAD.
Guidelines from the National Heart Lung and Blood Institute (2012) on cardiovascular disease in children and adolescents states that there is currently no medication therapy specific for elevated Lp(a), and similar to isolated low HDL–C levels, management may focus on addressing other risk factors and on more aggressively managing concomitant elevations of LDL–C, TG, and non-HDL–C. In adults, niacin will lower Lp(a) approximately 15 percent, but this has not been studied in children.

The Emerging Risk Factors Collaboration (Di Angelantonio, et al., 2012) found, in a study of individuals without known CVD, the addition of information on the combination of apolipoprotein B and A-I, lipoprotein(a), or lipoprotein-associated phospholipase A2 mass to risk scores containing total cholesterol and HDL-C led to slight improvement in CVD prediction. Individual records were available for 165,544 participants without baseline CVD in 37 prospective cohorts (calendar years of recruitment: 1968-2007) with up to 15,126 incident fatal or nonfatal CVD outcomes (10,132 CHD and 4994 stroke outcomes) during a median follow-up of 10.4 years (interquartile range, 7.6-14 years). The investigators assessed discrimination of CVD outcomes and reclassification of participants across predicted 10-year risk categories of low (<10%), intermediate (10%–<20%), and high (≥20%) risk. The addition of information on various lipid-related markers to total cholesterol, HDL-C, and other conventional risk factors yielded improvement in the model's discrimination: C-index change, 0.0006 (95% CI, 0.0002-0.0009) for the combination of apolipoprotein B and A-I; 0.0016 (95% CI, 0.0009-0.0023) for lipoprotein(a); and 0.0018 (95% CI, 0.0010-0.0026) for lipoprotein-associated phospholipase A2 mass. Net reclassification improvements were less than 1% with the addition of each of these markers to risk scores containing conventional risk factors. The investigators estimated that for 100,000 adults aged 40 years or older, 15,436 would be initially classified at intermediate risk using conventional risk factors alone. Additional testing with a combination of apolipoprotein B and A-I would reclassify 1.1%; lipoprotein(a), 4.1%; and
lipoprotein-associated phospholipase A2 mass, 2.7% of people to a 20% or higher predicted CVD risk category and, therefore, in need of statin treatment under Adult Treatment Panel III guidelines.

O'Donoghue et al (2014) evaluated the prognostic utility of Lp(a) in individuals with CAD. Plasma Lp(a) was measured in 6,708 subjects with CAD from 3 studies; data were then combined with 8 previously published studies for a total of 18,978 subjects. Across the 3 studies, increasing levels of Lp(a) were not associated with the risk of CV events when modeled as a continuous variable (odds ratio [OR]: 1.03 per log-transformed SD, 95 % CI: 0.96 to 1.11) or by quintile (Q5:Q1 OR: 1.05, 95 % CI: 0.83 to 1.34). When data were combined with previously published studies of Lp(a) in secondary prevention, subjects with Lp(a) levels in the highest quantile were at increased risk of CV events (OR: 1.40, 95 % CI: 1.15 to 1.71), but with significant between-study heterogeneity (p = 0.001). When stratified on the basis of LDL cholesterol, the association between Lp(a) and CV events was significant in studies in which average LDL cholesterol was greater than or equal to 130 mg/dl (OR: 1.46, 95 % CI: 1.23 to 1.73, p < 0.001), whereas this relationship did not achieve statistical significance for studies with an average LDL cholesterol less than 130 mg/dl (OR: 1.20, 95 % CI: 0.90 to 1.60, p = 0.21). The authors concluded that Lp(a) is significantly associated with the risk of CV events in patients with established CAD; however, there exists marked heterogeneity across trials. In particular, the prognostic value of Lp(a) in patients with low cholesterol levels remains unclear. The authors stated that “although the current study demonstrates that patients with established CAD who have a high level of Lp(a) are at an increased risk of subsequent MACE, the marked heterogeneity between studies raises questions regarding the value of Lp(a) as a clinically useful biomarker for risk assessment, particularly among patients with well-controlled LDL cholesterol. Moreover, although Lp(a) may directly contribute to CHD, there is currently insufficient evidence to suggest that Lp(a) levels above a discrete cut point should be used to guide therapy or that treatment will translate
into improved clinical outcomes”.

**Apo [Apolipoprotein] B Testing:**

An apolipoprotein is any of various proteins that combines with a lipid to form a lipoprotein, such as HDL or LDL. Apolipoproteins are important in the transport of cholesterol in the body and the regulation of the level of cholesterol in cells and blood. Apolipoprotein B (apo B) is the primary apolipoprotein of LDL, which is responsible for carrying cholesterol to tissues.

Each LDL particle has one molecule of apo B per particle. Therefore, the apo B concentration is an indirect measurement of the number of LDL particles, in contrast to LDL cholesterol, which is simply a measure of the cholesterol contained within the LDL. Because apo B is a marker for LDL particle number, the greater or higher the apo B level suggests an increased level of small, dense LDL particles which are thought to be especially atherogenic.

Guidelines from the ACC and the ADA recommend the use of apoB in persons at elevated cardiometabolic risk to assess whether additional intense interventions are necessary when LDL cholesterol goals are reached (Brunzell et al, 2008). According to these guidelines, high-risk persons are those with known CVD, diabetes, or multiple CVD risk factors (i.e., smoking, hypertension, family history of premature CVD). The American Association of Clinical Chemistry has issued similar recommendations regarding the use of apoB (Contois et al, 2009).

The INTERHEART study found the apo B:apo A-1 to be a stronger predictor of MI than their cholesterol counterparts (McQueen et al, 2008). In this study, 12,461 patients with acute MI from the world’s major regions and ethnic groups were compared with 14,637 age- and sex-matched controls to assess the contributions of various cardiovascular risk factors. Investigators obtained non-fasting blood samples from 9,345
cases and 12,120 controls and measured cholesterol fractions and apolipoproteins to determine their respective predictive values. Ratios were stronger predictors of MI than were individual components, and apolipoproteins were better predictors than their cholesterol counterparts. The apo B:apo A-1 ratio was the strongest predictor, with a population-attributable risk of 54 %, compared with risks of 37 % for LDL/HDL and 32 % for total cholesterol/HDL. A 1-standard-deviation increase in apo B:apo A-1 was associated with an odds ratio of 1.59 for MI, compared with 1.17 for an equivalent increase in total cholesterol/HDL. The results were similar for both sexes and across all ethnic groups and ages.

Apo B testing has not been validated as a tool for risk assessment in the general population. A study found that measuring apo B and apo A-I, the main structural proteins of atherogenic and antiatherogenic lipoproteins and particles, adds little to existing measures of CAD risk assessment and discrimination in the general population. van der Steeg et al (2007) measured apolipoprotein and lipid levels for 869 cases (individuals who developed fatal or nonfatal CAD) and 1,511 matched controls (individuals who remained CAD-free) over a mean follow-up of 6 years. Upon enrollment, participants were 45 to 79 years old and apparently healthy. Occurrence of CAD during follow-up was determined using a regional health authority database (hospitalizations) and U.K. Office of National Statistics records (deaths). The apo B:apo A-I ratio was associated with future CAD events independent of traditional lipid values, including total cholesterol:HDL cholesterol ratio (adjusted odds ratio, 1.85), and independent of the Framingham risk score (OR, 1.77). However, the apo B:apo A-I ratio did no better than lipid values in discriminating between individuals who would and would not develop CAD, and it added little to the predictive value of the Framingham risk score. In addition, this ratio incorrectly classified 41 % of cases and 50 % of controls.

A large, population-based, cohort study suggests that the apo B:apo A-1 ratio has little clinical utility in predicting
incident CHD in the general population, and that measuring total cholesterol and HDL appears to suffice to determine heart disease risk (Ingelsson et al, 2007). Investigators used a variety of techniques to evaluate the relative utility of apo B, apolipoprotein A-1 (apo A-1), serum total cholesterol, HDL cholesterol, LDL cholesterol, non-HDL cholesterol, and 3 lipid ratios in determining risk for CHD, as well as the relative ability of these measures to reclassify CHD risk. More than 3,300 middle-aged, white participants in the Framingham Offspring Study without CVD were followed for a median of 15 years. A total of 291 first CHD events occurred, 198 of them in men. In men, elevations in non-HDL cholesterol, apo B, total cholesterol:HDL ratio, LDL:HDL ratio, and apo B:apo A-1 ratio were all significantly associated with increased CHD risk to a similar degree. Elevated apo A-1 and HDL were likewise associated with reduced CHD risk. Women had results similar to those in men except that decreased apo A-1 was not significantly associated with incident CHD. In sex-specific analyses, elevated LDL and total cholesterol were not significantly associated with increased CHD risk in either men or women, perhaps owing to the lack of statistical power of these substudies. In men, total cholesterol:HDL and apo B:apo A-1 ratios both improved reclassification of 10-year risk for CHD; however, the difference between the two was not significant. In women, neither lipid ratio improved CHD risk reclassification.

Canadian Cardiovascular Society guidelines (2009, 2013) recommend apoB as the primary alternate target to LDL-C. The guidelines explain that, based on the available evidence, many experts have concluded that apoB is a better marker than LDL-C for the risk of vascular disease and a better index of the adequacy of LDL-lowering therapy than LDL-C. The guidelines also note that there now appears to be less laboratory error in the determination of apoB than LDL-C, particularly in patients with hypertriglyceridemia, and all clinical laboratories could easily and inexpensively provide standardized measurements of apoB. The guidelines state, however, that not all experts are fully convinced that apoB should be measured routinely and, in
any case, apoB is not presently being measured in most clinical laboratories. Consequently, a substantial educational effort for patients and physicians would be required for the most effective introduction of apoB into widespread clinical practice. The guidelines conclude that, despite these reservations, all would agree that physicians who wish to use apoB in their clinical care should be encouraged to do so. Furthermore, the present compromise approach represents a positive transitional phase in the assessment of lipid parameters to improve the prevention of CVD through the clinical measurement of apoB. The guidelines state that apoB target for high-risk subjects is less than 0.80 g/L.

Guidelines from the British Columbia Medical Services Commission (2008) states that apolipoprotein B (apoB) should be considered for follow-up testing in high-risk patients who are undergoing treatment for hypercholesterolemia (but not for other dyslipidemias). The guidelines state that other lipid tests are not required if using apoB for follow-up.

Guidelines from the American Association of Clinical Endocrinologists (2012) recommend apo B measurements to assess the success of LDL-C–lowering therapy. The guidelines note that LDL particle number as reflected by apo B is a more potent measure of cardiovascular disease (CVD) risk than LDL-C and LDL particle size (e.g., small, dense LDL).

A European consensus statement (2012) reported that, because apoB levels have so frequently been measured in outcome studies in parallel with LDL cholesterol, apoB can be substituted for LDL cholesterol, but it does not add further to the risk assessment. The guidelines found that, based on the available evidence, it appears that apoB is a similar risk marker to LDL cholesterol and a better index of the adequacy of LDL-lowering therapy. Also, there appears to be less laboratory error in the determination of apoB than LDL cholesterol, particularly in patients with hypertriglyceridemia, and laboratories could easily and inexpensively provide standardized measurements of apoB. The guideline stated, however, that apoB is not presently
being measured in most laboratories but, if measured, it should be less than 80 and less than 100 mg/dL for subjects with very high or high CVD risk, respectively.

Further study is needed to determine the usefulness of apolipoprotein B measurement as an adjunct to risk evaluation by routine lipid measurements in the general population. An assessment prepared for the Agency for Healthcare Research and Quality (Helfand, et al., 2009) concluded that "the contribution of ApoB ... to risk assessment for a first ASCVD event is uncertain at present."

There is emerging evidence of a relationship between apo B and stroke risk. Bhatia et al (2006) assessed the relationships between various lipid subfractions and ischemic stroke risk in a cohort of 261 patients after transient ischemic attack (TIA). During 10 years of follow-up, 45 patients experienced ischemic stroke. Apolipoprotein B (Apo B) and Apo B/Apo A1 ratio were the only predictors of stroke.

Standards of Care from the American Diabetes Association (2014) state that some experts recommend a greater focus on non–HDL cholesterol, apolipoprotein B (apoB), or lipoprotein particle measurements to assess residual CVD risk in statin-treated patients who are likely to have small LDL particles, such as people with diabetes, but it is unclear whether clinical management would change with these measurements.

A Working Group of the American Association for Clinical Chemistry (Cole, et al., 2013) found that, in most studies, both apoB and LDL particle number were comparable in association with clinical outcomes, and nearly equivalent in their ability to assess risk for cardiovascular disease. The Working Group stated that apo B appears to be the preferable biomarker for guideline adoption because of its availability, scalability, standardization, and relatively low cost.

The National Heart, Lung, and Blood Institute’s expert panel on integrated guidelines for cardiovascular health and risk
reduction in children and adolescents (2011) stated that “In terms of other lipid measurements: (i) at this time, most but not all studies indicate that measurement of apolipoprotein B (apoB) and apolipoprotein A-1 (apoA–1) for universal screening provides no additional advantage over measuring non-HDL–C, LDL–C, and HDL–C; (ii) measurement of lipoprotein(a) (Lp[a]) is useful in the assessment of children with both hemorrhagic and ischemic stroke; (iii) offspring of a parent with premature CVD and no other identifiable risk factors, elevations of apoB, apoA–1, and Lp(a) have been noted; and (iv) measurement of lipoprotein subclasses and their sizes by advanced lipoprotein testing has not been shown to have sufficient clinical utility in children at this time (Grade B”).

Also, UpToDate reviews on “Overview of the possible risk factors for cardiovascular disease” (Wilson, 2014a) and “Estimation of cardiovascular risk in an individual patient without known cardiovascular disease” (Wilson 2014b) do not mention the use of apolipoprotein A-1 (apoA-1) as a management tool.

The Institute for Clinical Systems Improvement’s clinical practice guideline on “Diagnosis and initial treatment of ischemic stroke” (Anderson et al, 2012) did not mention the measurements of markers of cholesterol production (lathosterol and desmosterol) and absorption (beta-sitosterol, campesterol, and cholestanol).

Also, UpToDate reviews on “Overview of the possible risk factors for cardiovascular disease” (Wilson, 2014a) and “Estimation of cardiovascular risk in an individual patient without known cardiovascular disease” (Wilson 2014b) do not mention measurements of markers of cholesterol production (lathosterol and desmosterol) and absorption (beta-sitosterol, campesterol, and cholestanol) as a management tools.

An Endocrine Society practice guideline (Berglund, et al., 2012) states that "The Task Force suggests that measurement of apolipoprotein B (apoB) or lipoprotein(a) [Lp(a)] levels can be of
value, whereas measurement of other apolipoprotein levels has little clinical value."

The Emerging Risk Factors Collaboration (Di Angelantonio, et al., 2012) found, in a study of individuals without known CVD, the addition of information on the combination of apolipoprotein B and A-I to risk scores containing total cholesterol and HDL-C led to slight improvement in CVD prediction. The investigators estimated that for 100,000 adults aged 40 years or older, 15,436 would be initially classified at intermediate risk using conventional risk factors alone. The investigators estimated that additional testing with a combination of apolipoprotein B and A-I would reclassify 1.1% of people to a 20% or higher predicted CVD risk category and, therefore, in need of statin treatment under Adult Treatment Panel III guidelines.

Guidelines from the American College of Cardiology and the American Heart Association (Goff, et al., 2014) state that "the contribution of ApoB ... to risk assessment for a first ASCVD event is uncertain at present."

**Apolipoprotein E (apo E) Testing:**

Apolipoprotein E (apo E) is a type of lipoprotein that is a major component of very low density lipoproteins (VLDL). Apo E is essential for the normal catabolism (breaking down) of triglyceride-rich lipoprotein constituents (components). A major function of VLDL is to remove excess cholesterol from the blood and carry it to the liver for processing.

Apo E is essential in the metabolism of cholesterol and triglycerides and helps to clear chyomicrons and very-low-density lipoproteins. Apo E has been studied for many years for its involvement in CVD. Apo E polymorphisms have functional effects on lipoprotein metabolism, and has been studied in disorders associated with elevated cholesterol levels and lipid derangements. The common isoforms of apolipoprotein E (apoE), E2, E3, and E4, have been found to be determinants of
plasma lipid concentrations, and 1 allele of the apoE gene, the epsilon4 (E4) allele is associated with an increased risk of coronary heart disease. In addition, the apoE4 allele is being investigated as a potential risk factor for Alzheimer's disease and stroke.

Several small studies and an earlier review have demonstrated variation in cholesterol levels and coronary disease risk associated with apo E isoforms. The literature on apo E and CVD was reviewed by Eichner et al (2002); the investigators concluded that the apo E genotype yields poor predictive values when screening for clinically defined atherosclerosis despite positive, but modest associations with plaque and coronary heart disease outcomes. The value of apo E testing in the diagnosis and management of CHD needs further evaluation.

One study found that smoking increases the risk of coronary heart disease in men of all apo E genotypes, but particularly in men carrying the epsilon4 allele. Humphries et al (2001) investigated whether the effect of smoking on coronary heart disease risk is affected by APOE genotype. The investigators enrolled 3,052 middle-aged men who were free of coronary heart disease for prospective cardiovascular surveillance in the second Northwick Park Heart Study (NPHSII). Compared with never-smokers, risk of coronary heart disease in ex-smokers was 1.34 (95% CI: 0.86 to 2.08) and in smokers it was 1.94 (1.25 to 3.01). This risk was independent of other classic risk factors. In never-smokers, risk was closely similar in men with different genotypes. Risk in men homozygous for the epsilon3 allele was 1.74 (1.10 to 2.77) in ex-smokers and 1.68 (1.01 to 2.83) in smokers, whereas in men carrying the epsilon4 allele risk was 0.84 (0.40 to 1.75) and 3.17 (1.82 to 5.50), respectively, with no significant differences in risk in the epsilon2 carriers. For the epsilon3 group, the genotype effect on risk was no longer significant after adjustment for classic risk factors (including plasma lipids). However, even after adjustment, smokers who were carriers of the epsilon4 allele, showed significantly raised risk of coronary heart disease compared with the non-smoking group (2.79,
1.59 to 4.91, epsilon4-smoking interaction p = 0.007). An accompanying editorial pointed out that it is important to determine how much of the variation in risk for CHD is attributable to the effects of apoE, in order to evaluate the importance of screening for apoE genotype (Wang and Mahaney, 2001).

Bennett et al (2007) conducted a meta-analysis to assess the relation of apo E genotypes to LDL cholesterol (LDL-C) and coronary disease risk. The researchers identified 82 studies of lipid levels (involving data on some 86,000 healthy participants) and 121 studies of coronary outcomes (involving data on some 38,000 cases and 83,000 controls) from both published and unreported sources. Pooling the lipid studies, researchers found a roughly linear relation toward increasing LDL-C levels when apo E genotypes were ordered 2/2, 2/3, 2/4, 3/3, 3/4, 4/4. Participants with the 2/2 genotype had LDL-C levels that were 31% lower than those with the 4/4 genotype. The associations were weaker between apo E alleles and triglyceride levels or HDL cholesterol levels. Turning to the coronary outcome studies, when the researchers used patients with the most common allele -- 3/3 -- as a reference, they found that carriers of the 2 allele had a 20% lower risk for coronary disease, while those with the 4 allele had a 6% increase in risk. Compared with individuals with the most common allele, those with the 2/2 genotype appear to have a 20% lower risk for coronary heart disease, while those with the 4/4 genotype appear to have a slightly higher risk. A commentator stated that these results are interesting, but the low prevalence of the 2 allele (about 7% in Western populations) and its association with the development of Parkinson disease make the consequences of these results -- and the utility and feasibility of routine screening -- uncertain (Foody, 2007).

Available evidence indicates that apo E genotype is a poor predictor of ischemic stroke. Sturgeon and colleagues examined whether apo E genotype alters the risk for ischemic stroke, as previous studies examining whether apo E genotype alters the
risk for stroke have yielded conflicting results. In this study, 14,679 individuals in the Atherosclerosis Risk in Communities (ARIC) study were genotyped for apo E. During more than 183,569 person-years of follow-up, 498 participants had an ischemic stroke. After stratifications by sex and race and adjustments for non-lipid risk factors for stroke, no significant relation between apo E genotype and stroke was identified, except for a lower risk associated with APOE-epsilon-2 compared with APOE-epsilon-3 in black women only. The investigators concluded that the apo E genotype is at most a weak factor for ischemic stroke.

The American Association of Clinical Chemistry (AACC, 2009) has stated that the test for apo E is not widely used and it’s clinical usefulness is still being researched. Guidelines from the American Association of Clinical Endocrinologists (2012) has a grade 2B recommendation that assessment of apo AI "may be useful in certain cases." The AACE guidelines state that a normal apo AI level in a patient with low HDL-C suggests the existence of an adequate number of HDL-C particles that contain less cholesterol and may be an indication of less risk.

**Homocysteine Testing:**

Homocysteine (Hcy) is an amino acid that is found normally in the body. Homocysteine is used by the body to make protein and to build and maintain tissue. Studies suggest that high blood levels of this substance may increase a person's chance of developing heart disease, stroke, and peripheral artery disease (PAD). It is believed that high levels of Hcy may damage arteries, may make blood more likely to clot, and may make blood vessels less flexible. It is also suggested that treatment consisting of high doses of folic acid, vitamins B6 and B12 decreases a patient's Hcy levels and thus decreases their risk of CVD. However, published study results in the medical literature are conflicting; therefore the usefulness of Hcy testing in reducing CVD risk and improving patient outcomes has not been demonstrated. ATP III noted the uncertainty about the strength of the relation between Hcy and CHD, a lack of clinical
trials showing that supplemental B vitamins will reduce risk for CHD, and the relatively low prevalence of elevated Hcy in the U.S. population.

In a structured evidence review, Hackam and Anand (2003) found moderate evidence that Hcy is an independent risk predictor of coronary heart, cerebrovascular and peripheral vascular disease. However, the authors found only minimal evidence that Hcy contributes incrementally to risk prediction. The authors also stated that it is unclear whether elevated Hcy is causal or simply a marker of atherosclerotic vascular disease. The authors found few, if any, controlled studies to evaluate risk-reduction strategies for these 4 factors. Hackman and Anand (2003) stated “whether homocysteine is causative in the pathogenesis of atherosclerosis, is related to other confounding cardiovascular risk factors, or is a marker of existing vascular disease will have to await the completion of a number of large, randomized controlled trials studying the effect of homocysteine-lowering vitamins on cardiovascular end points.”

An assessment by the Institute for Clinical Systems Improvement (ICSI, 2003) concluded that “the relevance of studies of [plasma homocysteine] as a risk factor for cardiovascular disease is unclear given the decreasing [plasma homocysteine] levels as a result of mandatory folic acid supplementation. It remains unproven whether lowered [plasma homocysteine] levels will result in reduced morbidity and mortality from cardiovascular disease.”

Prospective clinical studies have failed to demonstrate beneficial effects of Hcy-lowering therapy on CVD. An international randomized trial involved 5,522 patients with histories of documented vascular disease (coronary, cerebrovascular, or peripheral) or with diabetes plus another risk factor. Patients received either a combination pill (containing folic acid, vitamin B6, and vitamin B12 or placebo daily (HOPE 2 Investigators, 2006). After 5 years, mean Hcy levels were about 25% lower in the vitamin group than in the
placebo group. However, no significant difference was found between groups in the primary endpoint of MI, stroke, or cardiovascular death (18.8 % versus 19.8 %; p = 0.41) or in various secondary outcomes. Importantly, vitamin B supplementation did not benefit patients with the highest baseline Hcy levels or patients from countries without mandatory folate fortification of food.

In a secondary prevention randomized trial from Norway (Bonaa et al, 2006), 3,749 patients with MI during the preceding 7 days received vitamin B supplements or placebo. During an average follow-up of 3 years, vitamin supplementation conferred no benefit for any clinical outcome.

A randomized controlled clinical trial found no effect of treatment with folic acid, vitamin B12 and vitamin B6 for secondary prevention in patients with coronary artery disease or aortic valve stenosis (Ebbing et al, 2008). The researchers reported on a randomized, double-blind controlled trial conducted in the 2 university hospitals in western Norway in between 1999 and 2006. A total of 3,096 adult participants undergoing coronary angiography were randomized. At baseline, 59.3 % had double- or triple-vessel disease, 83.7 % had stable angina pectoris, and 14.9 % had acute coronary syndromes. Study participants were randomly assigned to 1 of 4 groups receiving daily oral treatment with folic acid plus vitamin B12 and vitamin B6; folic acid plus vitamin B12; vitamin B6 alone; or placebo (n = 780). The primary end point of this study was a composite of all-cause death, non-fatal acute MI, acute hospitalization for unstable angina pectoris, and non-fatal thromboembolic stroke. Mean plasma total Hcy concentration was reduced by 30 % after 1 year of treatment in the groups receiving folic acid and vitamin B12. The trial was terminated early because of concern among participants due to preliminary results from a contemporaneous Norwegian trial suggesting adverse effects from the intervention. During a median 38 months of follow-up, the primary end point was experienced by a total of 422 participants (13.7 %): 219 participants (14.2 %) receiving folic acid/vitamin B12 versus 203 (13.1 %) not
receiving such treatment (HR, 1.09; 95% CI: 0.90 to 1.32; p = 0.36) and 200 participants (13.0%) receiving vitamin B6 versus 222 (14.3%) not receiving vitamin B6 (HR, 0.90; 95% CI: 0.74 to 1.09; p = 0.28). The investigators concluded that this trial did not find an effect of treatment with folic acid, vitamin B12 or vitamin B6 on total mortality or cardiovascular events. The researchers concluded that "[o]ur findings do not support the use of B vitamins as secondary prevention in patients with coronary artery disease."

A randomized trials among women with and without pre-existing CVD failed to support benefits of B-vitamin supplementation on cardiovascular risk (Albert et al, 2008). Within an ongoing RCT of antioxidant vitamins, 5,442 women who were U.S. health professionals aged 42 years or older, with either a history of CVD or 3 or more coronary risk factors, were enrolled in a randomized, double-blind, placebo-controlled trial to receive a combination pill containing folic acid, vitamin B6, and vitamin B12 or a matching placebo, and were treated for 7.3 years from April 1998 through July 2005. The primary endpoint of the study was a composite outcome of MI, stroke, coronary re-vascularization, or CVD mortality. Compared with placebo, a total of 796 women experienced a confirmed CVD event (406 in the active group and 390 in the placebo group). Patients receiving active vitamin treatment had similar risk for the composite CVD primary end point (226.9/10,000 person-years versus 219.2/10,000 person-years for the active versus placebo group; relative risk (RR), 1.03; 95% CI: 0.90 to 1.19; p = 0.65), as well as for the secondary outcomes including MI (34.5/10,000 person-years versus 39.5/10,000 person-years; RR, 0.87; 95% CI: 0.63 to 1.22; p = 0.42), stroke (41.9/10,000 person-years versus 36.8/10,000 person-years; RR, 1.14; 95% CI: 0.82 to 1.57; p = 0.44), and CVD mortality (50.3/10,000 person-years versus 49.6/10,000 person-years; RR, 1.01; 95% CI: 0.76 to 1.35; p = 0.93). In a blood substudy, geometric mean plasma Hcy level was decreased by 18.5% (95% CI: 12.5% to 24.1%; p < 0.001) in the active group (n = 150) over that observed in the placebo group (n = 150), for a difference of 2.27 micromol/L (95% CI: 1.54 to 2.96
micromol/L). The researchers concluded that, after 7.3 years of treatment and follow-up, a combination pill of folic acid, vitamin B6, and vitamin B12 did not reduce a combined endpoint of total cardiovascular events among high-risk women, despite significant Hcy lowering.

Despite the biological plausibility of lower plasma Hcy levels improving endothelial function, a RCT showed no benefit, and actual harm, from B-vitamin supplementation in patients with diabetic nephropathy (House et al, 2010). Hyperhomocysteinemia is frequently observed in patients with diabetic nephropathy. B-vitamin therapy (folic acid, vitamin B(6), and vitamin B(12)) has been shown to lower the plasma concentration of Hcy. In order to determine whether B-vitamin therapy can slow progression of diabetic nephropathy and prevent vascular complications, investigators conducted a multi-center, randomized, double-blind, placebo-controlled trial (Diabetic Intervention with Vitamins to Improve Nephropathy [DIVINE]) at 5 university medical centers in Canada between May 2001 and July 2007 (House et al, 2010). The study involved 238 participants who had type 1 or 2 diabetes and a clinical diagnosis of diabetic nephropathy. Subjects were randomly assigned to receive B vitamins containing folic acid, vitamin B6, and vitamin B12, or matching placebo. The main outcome measure was a change in radionuclide glomerular filtration rate (GFR) between baseline and 36 months. Secondary outcomes were dialysis and a composite of MI, stroke, re-vascularization, and all-cause mortality. Plasma total Hcy was also measured. The mean (SD) follow-up during the trial was 31.9 (14.4) months; enrollment was ended early by the data and safety monitoring board. At 36 months, the mean decrease in GFR was significantly greater in B-vitamin recipients than in non-recipients, even though plasma Hcy levels declined substantially in treated patients and rose in controls. Treated patients also incurred roughly double the risk for adverse cardiovascular events as did controls. At 36 months, radionuclide GFR decreased by a mean (SE) of 16.5 (1.7) mL/min/1.73 m(2) in the B-vitamin group compared with 10.7 (1.7) mL/min/1.73 m(2) in the placebo group (mean
difference, -5.8; 95 % CI: -10.6 to -1.1; P = .02). There was no difference in requirement of dialysis (HR, 1.1; 95 % CI: 0.4 to 2.6; p = 0.88). The composite outcome occurred more often in the B-vitamin group (HR, 2.0; 95 % CI: 1.0 to 4.0; p = 0.04). Plasma total Hcy decreased by a mean (SE) of 2.2 (0.4) micromol/L at 36 months in the B-vitamin group compared with a mean (SE) increase of 2.6 (0.4) micromol/L in the placebo group (mean difference, -4.8; 95 % CI: -6.1 to -3.7; p < 0.001, in favor of B vitamins). The authors concluded that, among patients with diabetic nephropathy, high doses of B vitamins compared with placebo resulted in a greater decrease in GFR and an increase in vascular events. Commenting on this study, Schwenk (2010) stated, "[g]iven that most other trials also have shown that B-vitamin supplementation does not prevent stroke and CV disease, such supplements should be avoided unless patient subgroups that derive benefit are identified in future clinical trials."

A long-term RCT involving survivors of MI found that substantial long-term reductions in blood Hcy levels with folic acid and vitamin B12 supplementation did not have beneficial effects on vascular outcomes (Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) Collaborative Group, 2010). In this double-blind RCT of 12,064 survivors of MI in secondary care hospitals in the United Kingdom between 1998 and 2008, subjects were randomized to 2 mg folic acid plus 1 mg vitamin B12 daily or to matching placebo. Study endpoints were first major vascular event, defined as major coronary event (coronary death, MI, or coronary re-vascularization), fatal or non-fatal stroke, or non-coronary re-vascularization. The investigators reported that allocation to the study vitamins reduced Hcy by a mean of 3.8 µmol/L (28 %). During 6.7 years of follow-up, major vascular events occurred in 1,537 of 6,033 participants (25.5 %) allocated folic acid plus vitamin B12 versus 1,493 of 6,031 participants (24.8 %) allocated placebo (risk ratio [RR], 1.04; 95 % CI: 0.97 to 1.12; p = 0.28). The investigators found no apparent effects on major coronary events (vitamins, 1,229 [20.4 %], versus placebo, 1,185 [19.6 %]; RR, 1.05; 95 % CI: 0.97
to 1.13), stroke (vitamins, 269 [4.5 %], versus placebo, 265 [4.4 %]; RR, 1.02; 95 % CI: 0.86 to 1.21), or non-coronary revascularizations (vitamins, 178 [3.0 %], versus placebo, 152 [2.5 %]; RR, 1.18; 95 % CI: 0.95 to 1.46). The investigators did not find significant differences in the numbers of deaths attributed to vascular causes (vitamins, 578 [9.6 %], versus placebo, 559 [9.3 %]) or non-vascular causes (vitamins, 405 [6.7 %], versus placebo, 392 [6.5 %]). An accompanying commentary by Schwenk (2010) stated: "These results, and those of the seven prior major trials, should end what seems to be an unjustified persistence by many clinicians to recommend folate supplementation to prevent CV disease. Clinical efforts should focus on modification of CV risk factors, for which evidence supports improved outcomes."

These results are consistent with earlier RCTs of Hcy lowering therapy for CVD. In a multi-center double-blind randomized study, Toole et al (2004) enrolled 3,680 patients with non-disabling, non-embolic ischemic strokes and total Hcy levels above the 25th percentile for the North American stroke population. Patients received either high-doses of Hcy-lowering vitamins (2.5 mg folic acid, 25 mg pyridoxine, and 0.4 mg cobalamin) or low doses that would not be expected to lower Hcy significantly (20 µg, 200 µg, and 6 µg, respectively). During 2 years of follow-up, mean total Hcy decreased from 13.4 µmol/L to about 11 µmol/L in the high-dose group and changed only minimally in the control group. However, no reductions were noted in rates of recurrent stroke, coronary events, or death. Even in the subgroup with the highest Hcy levels, high-dose therapy was ineffective.

In an open-label, prospective trial from the Netherlands, Liem et al (2003) randomized 593 consecutive outpatients with CAD to folic acid or to standard care. All had been taking statins for at least 3 months. The 2 groups had similar baseline characteristics, including mean plasma Hcy levels of 12 µmol/L. By 3 months, Hcy levels had decreased among folic-acid recipients (by 18%) but not among controls. By a mean follow-up of 24 months, clinical vascular events (i.e., death, MI,
stroke, invasive coronary procedures, vascular surgery) had occurred at similar rates in folic-acid (12.3%) and standard-care (11.2%) recipients; the similarity also was evident among patients in the highest quartile of baseline Hcy level (greater than 13.7 µmol/L). In multi-variate analysis, poor creatinine clearance was a more important cardiovascular risk factor than elevated Hcy level was.

Routine testing for Hcy is also not supported in persons with venous thromboembolism. In a secondary analysis of a previously published multi-national RCT designed to assess the effect of Hcy-lowering therapy on the risk for arterial disease (Ray et al, 2007), investigators studied whether daily folate (2.5 mg) and vitamins B6 (50 mg) and B12 (1 mg) affected the risk for symptomatic deep venous thrombosis or pulmonary embolism. Subjects were 5,522 adults (age 55 years and older) with arterial vascular disease, diabetes, and at least 1 other CVD risk factor. During a mean follow-up of 5 years, Hcy levels decreased more in the vitamin-therapy group than in the placebo group. However, the incidence of venous thromboembolism did not differ between the vitamin-therapy and placebo groups, both overall and among the quartile with the highest Hcy levels (i.e., greater than 13.8 µmol/L) at baseline.

These results were similar to an earlier secondary prevention trial of Hcy for venous thromboembolism (VTE). In the first randomized trial of Hcy therapy to prevent recurrent VTE, den Heijer et al (2007) enrolled 701 patients with recent VTE (either proximal deep-vein thrombosis or pulmonary embolism), but without major predisposing risk factors such as recent surgery or immobilization. At baseline, 50% the patients had hyper-homocysteinemia (mean, 15.5 µmol/L), and 50% had normal levels (mean, 9.0 µmol/L). Patients were randomized to receive a B-vitamin supplement (5 mg folic acid, 0.4 mg B12, and 50 mg B6) or placebo, in addition to standard anti-coagulation. During 2.5 years of follow-up, the overall incidence of recurrent VTE was not significantly different in the B-vitamin and placebo groups (5.4 % versus. 6.4 %). In hyper-homocysteinemic
patients, the incidence of recurrent venous thromboembolism was non-significantly higher in B-vitamin recipients than in placebo recipients (6.7 % versus 6.0 %); in those with normal Hcy, the incidence of recurrent VTE was non-significantly lower in B-vitamin recipients (4.1 % versus 7.0 %). The authors noted that their study might have been under-powered to detect a small beneficial effect. However, they also speculate that Hcy's observed epidemiologic association with venous thromboembolism might in fact be mediated by some other thrombophilic factor that is correlated with Hcy.

An American Heart Association Science Advisory (Malinow et al., 1999) has concluded: "Although there is considerable epidemiological evidence for a relationship between plasma homocyst(e)ine and cardiovascular disease, not all prospective studies have supported such a relationship .... Until results of controlled clinical trials become available, population-wide screening is not recommended.... Such treatment (supplemental vitamins) is still considered experimental, pending results from intervention trials showing that homocyst(e)ine lowering favorably affects the evolution of arterial occlusive diseases."

A consensus statement from the ACC and the ADA (Brunzell et al., 2008) reported that Hcy testing has been evaluated to determine its prognostic significance in CVD. However, the independent predictive value of Hcy testing and its clinical utility are unclear.

The National Academy of Clinical Biochemistry (Cooper and Pfeiffer, 2009) stated that "we conclude that the clinical application of Hcy measurement for risk assessment of primary prevention of CVD is currently uncertain."

An assessment prepared for the Agency for Healthcare Research and Quality (Helfand, et al., 2009) found that "homocysteine ... probably provide[s] independent information about coronary heart disease risk, but data about their prevalence and impact when added to Framingham risk score in
intermediate-risk individuals are limited."

The U.S. Preventive Services Task Force (USPSTF, 2009) stated that there is insufficient evidence to recommend the use of Hcy to screen asymptomatic individuals with no history of CHD to prevent CHD events.

A statement issued by the American Heart Association (AHA, 2010, 2014) states that the AHA does not consider high Hcy levels in the blood to be a major risk factor for cardiovascular disease. The AHA states that a causal link between Hcy levels and atherosclerosis has not been established.


Guidelines from the American Association of Clinical Endocrinology (2012) do not recommend the routine measurement of homocysteine, noting that several studies have shown no benefit to intervention.

Guidelines from the Royal Australian College of General Practitioners (2012) reported that the value of homocysteine as a risk factor for CHD is uncertain and published RCTs show no evidence of benefit by lowering levels of homocysteine.

Summarizing the evidence for use of homocysteine, a European consensus guideline (2012) stated that homocysteine has shown precision as an independent risk factor for cardiovascular disease. The guidelines state that magnitude of homocysteine's effect on risk is modest, and consistency is often lacking, mainly due to nutritional, metabolic (e.g. renal disease), and lifestyle confounders. The guidelines note that, in addition, intervention studies using B vitamins to reduce plasma homocysteine have proven inefficient in reducing risk of cardiovascular disease. The guidelines conclude that, together with the cost of the test, homocysteine remains a "second-line" marker for cardiovascular disease risk estimation. The
guidelines include a strong recommendation that homocysteine should not be measured to monitor cardiovascular disease risk prevention. The guidelines include a weak recommendation that homocysteine may be measured as part of a refined risk assessment in patients with an unusual or moderate CVD risk profile.

Veeranna et al (2011) examined if adding Hcy to a model-based on traditional CVD risk factors improves risk classification. These researchers performed a post-hoc analysis of the MESA (Multi-Ethnic Study of Atherosclerosis) and NHANES III (National Health and Nutrition Examination Survey III) datasets. Homocysteine was used to predict composite CVD and hard CHD events in the MESA study and CVD and CHD mortality in the NHANES III survey using adjusted Cox-proportional hazard analysis. Re-classification of CHD events was performed using a net reclassification improvement (NRI) index with a Framingham risk score (FRS) model with and without Hcy. Homocysteine level (greater than 15 μmol/L) significantly predicted CVD (adjusted hazard ratio [aHR]: 1.79, 95 % CI: 1.19 to 1.95; p = 0.006) and CHD events (aHR: 2.22, 95 % CI: 1.20 to 4.09; p = 0.01) in the MESA trial and CVD (aHR: 2.72, 95 % CI: 2.01 to 3.68; p < 0.001) and CHD mortality (aHR: 2.61, 95 % CI: 1.83 to 3.73; p < 0.001) in the NHANES III, after adjustments for traditional risk factors and CRP. The level of Hcy, when added to FRS, significantly re-classified 12.9 % and 18.3 % of the overall and 21.2 % and 19.2 % of the intermediate-risk population from the MESA and NHANES cohorts, respectively. The categoryless NRI also showed significant re-classification in both MESA (NRI: 0.35, 95 % CI: 0.17 to 0.53; p < 0.001) and NHANES III (NRI: 0.57, 95 % CI: 0.43 to 0.71; p < 0.001) datasets. The authors concluded that from these 2 disparate population cohorts, they found that addition of Hcy level to FRS significantly improved risk prediction, especially in individuals at intermediate-risk for CHD events.

In an editorial that accompanied the afore-mentioned study, Mangoni and Woodman (2011) stated that "[i]f Hcy is to be used as a screening tool in primary prevention, it is imperative
that further trials are conducted in low- and intermediate-risk patients without previous CVD. Only then can the real value of measuring Hcy as a nontraditional CVD risk factor or risk marker be quantified".

**Intermediate Density Lipoproteins**

Lipoprotein remnants testing measures triglyceride-rich lipoproteins that include intermediate density lipoproteins (IDL) and VLDL. It is proposed that lipoprotein remnants penetrate arterial walls more easily than larger lipoproteins and may be independent risk factors for CVD.

Data from the Framingham Study have suggested that remnant-like particle cholesterol (RLP-C) (intermediate density lipoproteins) is an independent risk factor for CVD in women, and studies have shown that hormone therapy can lower RLP-C levels in healthy post-menopausal women.

The Women's Angiographic Vitamin and Estrogen (WAVE) trial (Bittner et al, 2004) examined whether hormone therapy can reduce RLP-C and RLP-triglyceride (TG) levels in women with coronary artery disease, and whether these factors predict disease progression. WAVE was a randomized, placebo-controlled, clinical trial of hormone therapy (conjugated equine estrogen or estrogen plus medroxyprogesterone acetate) and antioxidants in 423 post-menopausal women with angiographic coronary disease; follow-up angiography at 2.8 years showed no benefit with hormone therapy or antioxidants, and no interaction between the two. The WAVE investigators also measured RLP-C and RLP-TG levels in a subset of 397 women. Mean RLP values among the WAVE participants were very high, corresponding to the 90th percentiles in the Framingham cohort. In multi-variate analyses, RLP-C and RLP-TG levels were not related to waist-hip ratio, body mass index (BMI), smoking status, or use of lipid-lowering agents. Compared with placebo, hormone therapy did not significantly reduce RLP levels. Neither baseline RLP levels nor changes in the levels predicted angiographic findings at the
end of the study.

The National Cholesterol Education Program Adult Treatment Panel III (ATPIII) Guidelines (2002) state that lipoprotein remnants, including intermediate density lipoproteins (IDLs), as well as very-low-density lipoproteins (VLDL) and small density lipoproteins, have been shown to be atherogenic through several lines of evidence. According to ATPIII, “prospective studies relating various measures to CHD risk are limited, and measurement with specific assays cannot be recommended for routine practice.” The ATPIII panel concluded, however, that the most readily available method of measuring atherogenic triglyceride-rich lipoproteins is measurement of VLDL. A consensus statement by the ACC and the ADA (Brunzell et al, 2008) noted that, although small dense LDL has been shown to be particularly atherogenic, the association of small LDL and cardiovascular disease may simply reflect the increased number of LDL particles in patients with small LDL.

According to guidelines from the American College of Cardiology and the American Heart Association (2010), measurement of lipid parameters, including particle size and density, beyond a standard fasting lipid profile is not recommended for cardiovascular risk assessment in asymptomatic adults.

**HDL Subspecies:**

Lipoprotein subfraction testing is testing that separates two of the commonly measured lipoprotein fractions, HDL and LDL, into subclasses based on their size, density and/or electrical charge. HDL subclass testing is suggested to provide information regarding CVD risk when utilized with standard lipoprotein tests, such as total cholesterol, HDL and LDL testing.

HDL comprises several components and subfractions that also have been related to CHD risk. While HDL cholesterol is the risk indicator most often used, HDL subfractions (lipoprotein AI
(LpAI) and lipoprotein AI/AII (LpAI/AII) and/or HDL3 and HDL2) have also been used for risk prediction. ATPIII concluded, however, that the superiority of HDL subspecies over HDL cholesterol has not been demonstrated in large, prospective studies. Consequently, ATPIII did not recommend the routine measurement of HDL subspecies in CHD risk assessment. A consensus statement by the ACC and the ADA (Brunzell et al, 2008) state that measurements of HDL subfractions appear to provide little clinical value beyond measurements of HDL cholesterol.

**LDL Subspecies (LDL Particle Sizes) and LDL Particle Number:**

LDL subclass testing is suggested as part of an overall risk assessment for CVD, this test measures the cholesterol content of lipoprotein particles in the blood and determines the LDL particle size and/or density pattern.

Density gradient ultracentrifugation (Vertical Autoprofile (VAP) test) measures the relative distribution of cholesterol within various lipoprotein subfractions, quantifying the cholesterol content of VLDL, IDL, LDL, lipoprotein(a), and HDL subclasses (Mora, 2009). The VAPI also determines the predominant LDL size distribution (eg, A, AB, or B phenotype) but does not provide concentrations of the lipoprotein particles themselves. ApoB is also provided, although it is not measured directly. Some labs offer vertical lipoprotein particle (VLP) technology included with the VAP test to further analyze CVD risk. The VLP technology purportedly reports a true particle number (LDL-P), a proposed biomarker for increased risk of heart disease and stroke.

**Nuclear Magnetic Resonance (NMR) Spectroscopy**

(Normag) is based on the concept that each lipoprotein particle in plasma of a given size has its own characteristic lipid methyl group nuclear magnetic resonance (NMR) signal (Mora, 2009). Particle concentrations of lipoprotein subfractions of different size are obtained from the measured amplitudes of their lipid methyl group NMR signals. Lipoprotein particle sizes
are then derived from the sum of the diameter of each subclass multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal. The NMR LipoProfile simultaneously quantifies lipoprotein concentrations of VLDL, IDL, LDL, and HDL particles and their subfractions, each expressed as a lipoprotein particle concentration (number of particles per liter) or as an average particle size for each of VLDL, LDL, and HDL.

The gradient gel electrophoresis method determines the distribution of LDL size phenotype by proprietary segmented polyacrylamide gradient gels, which separate lipoproteins in a gradient gel on the basis of their size and, to a lesser extent, their charge (Mora, 2009). Pattern A corresponds to large LDL particles; B to small, dense LDL particles; and AB to an intermediate phenotype. This method gives the relative, or predominant, distribution of lipoprotein particles as determined by the predominant peak particle size.

LDL gradient gel electrophoresis (GGE) has been promoted as an important determinant of CHD risk, and as a guide to drug and diet therapy in patients with established CAD. The measurement of LDL subclass patterns may be useful in elucidating possible atherogenic dyslipemia in patients who have no abnormalities in conventional measurement (total cholesterol, HDL, LDL, and triglycerides). However, the therapeutic usefulness of discovering such subclass abnormalities has not been substantiated.

Ion mobility analysis measures both the size and concentrations of lipoprotein particle subclasses on the basis of gas-phase differential electric mobility.

A number of studies have reported that both larger low-density lipoprotein (LDL) particle size and smaller LDL particle sizes are more atherogenic than intermediate-sized particles, and these particles at the extremes of LDL size may be associated with coronary heart disease (CHD) risk. It is thought that LDL subspecies at both extremes of LDL size and density distribution
have a reduced LDL receptor affinity.

Musunuru, et al. (2009) tested whether combinations of lipoprotein subfractions independently predict cardiovascular disease in a prospective cohort of 4594 initially healthy men and women (the Malmö Diet and Cancer Study, mean follow-up 12.2 years, 377 incident cardiovascular events). Plasma lipoproteins and lipoprotein subfractions were measured at baseline with a novel high-resolution ion mobility technique. Principal component analysis (PCA) of subfraction concentrations identified 3 major independent (ie, zero correlation) components of CVD risk, one representing LDL-associated risk, a second representing HDL-associated protection, and the third representing a pattern of decreased large HDL, increased small/medium LDL, and increased triglycerides. The last corresponds to the previously described "atherogenic lipoprotein phenotype." Several genes that may underlie this phenotype-CETP, LIPC, GALNT2, MLXIPL, APOA1/A5, LPL-are suggested by SNPs associated with the combination of small/medium LDL and large HDL. The investigators concluded that principal component analysis on lipoprotein subfractions yielded three independent components of CVD risk. Genetic analyses suggest these components represent independent mechanistic pathways for development of CVD.

ATPIII stated that although the presence of small LDL particles has been associated with an increased risk of CHD, the extent to which small LDL particles predict CHD independent of other risk factors is “controversial.” It has been argued by Campos et al (2002), based on epidemiologic evidence, that the relationship between small LDL and CHD found in some studies is probably due to its correlation with other lipoprotein risk factors, and that small LDL is not an independent risk factor for CHD.

Campos et al (2002) demonstrated in a prospective cohort study that large LDL size is a potential statistically significant predictor of coronary events. Large LDL particles are thought to
be large because of high cholesterol ester content. However, Campos reported that the relationship between LDL particle size and coronary events was not present among members of the cohort who were treated with pravastatin, perhaps because pravastatin acts by reducing the size of LDL particles. The author concluded that identifying patients on the basis of LDL size may not be useful clinically, since effective treatment for elevated LDL cholesterol concentrations also effectively treats risk associated with large LDL.

Commenting on LDL particle size, a consensus statement from the ACC and the ADA stated: "The size of LDL particles can also be measured. As small dense LDL particles seem to be particularly atherogenic, assessment of particle size has intuitive appeal. Both LDL particle concentration and LDL size are important predictors of CVD. However, the Multi-Ethnic Study of Atherosclerosis suggested that on multi-variate analyses, both small and large LDL were strongly associated with carotid intima-media thickness [Mora et al, 2007], while the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) showed that both were significantly related to coronary heart disease (CHD) events [Otvos et al, 2006]. The association of small LDL and CVD may simply reflect the increased number of LDL particles in patients with small LDL. Hence, it is unclear whether LDL particle size measurements add value to measurement of LDL particle concentration" (Brunzell et al, 2008).

The ACC/ADA consensus statement recommended ApoB measurement over measurement of particle number with NMR (Brunzell et al, 2008): "Limitations of the clinical utility of NMR measurement of LDL particle number or size include the facts that the technique is not widely available and that it is currently relatively expensive. In addition, there is a need for more independent data confirming the accuracy of the method and whether its CVD predictive power is consistent across various ethnicities, ages, and conditions that affect lipid metabolism."

An assessment by the California Technology Assessment Forum
(CTAF) (Walsh, 2008) of LDL particle number as assessed by NMR spectroscopy concluded that this test did not meet CTAF’s assessment criteria. The CTAF assessment stated that there were no studies addressing whether or not treated LDL particle levels affected clinical outcomes.

A systematic evidence review of LDL subfractions, including the methods of gradient gel electrophoresis, NMR spectroscopy, and ultra-centrifugation, prepared for the Federal Agency for Healthcare Research and Quality (AHRQ) concluded that "the data do not adequately answer the question of how strongly LDL subfraction information is associated with CVD [cardiovascular disease], in relation to other known and putative risk factors. In summary, none of the LDL subfraction measurements have definitively been demonstrated to add to the ability to discriminate between individuals who are at higher versus lower risks of cardiovascular events compared to commonly used predictors, such as LDL and HDL cholesterol" (Balk et al, 2008). The AHRQ report stated that it has yet to be determined if cardiac disease risk assessment and treatment decisions would be improved by adding LDL subfraction (subclass) measurements (Balk et al, 2008).

An assessment by the National Academy of Clinical Biochemistry (Wilson et al, 2009) concluded that lipoprotein subclasses have been shown to be related to the development of initial CHD events, but the data analyses of existing studies are generally not adequate to show added benefit over standard risk assessment for primary prevention. The assessment found that there are also insufficient data that measurement of lipoprotein subclasses over time is useful to evaluate the effects of treatments. The assessment also noted that several methods are available to assess lipoprotein subclasses, and that standardization is needed for this technology.

The NACB assessment on LDL particle concentration and subclasses (including measurement by gradient gel electrophoresis) (Wilson et al, 2009) concluded: "Lipoprotein
subclasses, especially the number or concentration of small, dense LDL particles, have been shown to be related to the development of initial CHD events, but the data analyses of existing studies are generally not adequate to show added benefit over standard risk assessment for primary prevention."

There is inadequate evidence that LDL subclassification by electrophoresis improves outcomes of patients with cardiovascular disease. According to the guidelines of the National Cholesterol Education Program, electrophoretic methods “cannot be recommended as procedures of choice for measuring LDL-cholesterol.”

Furthermore, guideline from the National Academy of Clinical Biochemistry (Myers, 2009) does not support LDL subclass testing.

According to guidelines from the American College of Cardiology and the American Heart Association (2010), measurement of lipid parameters, including particle size and density, beyond a standard fasting lipid profile is not recommended for cardiovascular risk assessment in asymptomatic adults. Guidelines from the Canadian Cardiovascular Society (2013) recommend measurement of ApoB or non-HDL-C as alternative targets, and make no recommendation for use of other measures of lipid particle number.

Guidelines from the National Heart Lung and Blood Institute (2012) on cardiovascular disease in children and adolescents concluded that measurement of lipoprotein subclasses and their sizes by advanced lipoprotein testing has not been shown to have sufficient clinical utility in children at this time. The guidelines state that the plasma levels of VLDL-C, LDL-C, and HDL-C subclasses and their sizes have been determined in children and adolescents by nuclear magnetic resonance spectroscopy and by vertical-spin density-gradient ultracentrifugation in research studies, but cutpoints derived from these methods for the diagnosis and treatment of
dyslipidemia in youths are not currently available.

Guidelines on prevention of cardiovascular disease in women from the American Heart Association (Mosca, et al., 2011) state that the role that novel CVD risk biomarkers, including advanced lipid testing, should play in risk assessment and in delineation of appropriate preventive interventions is not yet well defined.

A special report of an AACC Working Group on apoB and NMR Lipoprofile for measuring particle number (Cole, et al., 2013) concluded: "Currently, in the opinion of this Working Group on Best Practices, apo B appears to be the preferred biomarker for guideline adoption because of its widespread availability, scalability, standardization, and relatively low cost."

Standards of Care from the American Diabetes Association (2013) state that some experts recommend a greater focus on non–HDL cholesterol, apolipoprotein B (apoB), or lipoprotein particle measurements to assess residual CVD risk in statin-treated patients who are likely to have small LDL particles, such as people with diabetes, but it is unclear whether clinical management would change with these measurements.

An Endocrine Society Clinical Practice Guideline on hypertriglyceridemia (Brunzell, et al., 2012) states that "The Task Force recommends against the routine measurement of lipoprotein particle heterogeneity in patients with hypertriglyceridemia."

According to guidelines from the American College of Cardiology and the American Heart Association (2010), measurement of lipid parameters, including particle size and density, beyond a standard fasting lipid profile is not recommended for cardiovascular risk assessment in asymptomatic adults.

Guidelines on prevention of cardiovascular disease in women from the American Heart Association (Mosca, et al., 2011) state
that the role that novel CVD risk biomarkers, including advanced lipid testing, should play in risk assessment and in delineation of appropriate preventive interventions is not yet well defined.

Guidelines from the National Heart Lung and Blood Institute (2012) on cardiovascular disease in children and adolescents concluded that measurement of lipoprotein subclasses and their sizes by advanced lipoprotein testing has not been shown to have sufficient clinical utility in children at this time.

Angiotensin Gene:

Angiotensin gene polymorphisms have been associated with CVD risk and certain forms of hypertension. Certain AGT polymorphisms have been associated with responsiveness of BP to sodium restriction and ACE inhibitors, so that analysis of the AGT gene may have the potential to help individualize therapy by predicting patients’ responsiveness to certain anti-hypertensive interventions. CardiaRisk AGT from Myriad Genetics Laboratories is a laboratory test that analyzes the angiotensinogen gene. The value of analyzing angiotensin gene polymorphisms in altering the management and improving outcomes of patients has not been demonstrated in prospective clinical studies.

Fibrinogen:

Fibrinogen is a circulating glycoprotein in the blood that helps blood clot. Too much fibrinogen may promote excessive clumping of platelets. This can cause clots to form in an artery, which may lead to heart attack or stroke. Fibrinogen has been suggested as a possible indicator of inflammation that accompanies atherosclerosis.

Fibrinogen acts at the final step in the coagulation response to vascular and tissue injury, and epidemiological data support an independent association between elevated levels of fibrinogen and cardiovascular morbidity and mortality.
In a structured evidence review, Hackman and Anand (2003) found moderate evidence that fibrinogen is an independent risk predictor for atherosclerotic disease (CHD, cerebrovascular disease, and peripheral vascular disease). However, they found minimal evidence that fibrinogen is an incremental risk predictor. Hackam and Anand (2003) identified only 1 study that examined the additive yield of screening for fibrinogen. The authors noted that precise and validated tests are not available for fibrinogen. In addition, they concluded that it is unclear whether fibrinogen is causal or are simply markers of atherosclerotic vascular disease. The investigators found, few, if any, controlled studies evaluating risk-reduction strategies for fibrinogen or any of the other novel risk factors that they evaluated. The investigators concluded that “clinical trials are necessary before it can be determined whether fibrinogen has a causal role in atherothrombosis or is merely a marker of the degree of vascular damage taking place.”

A consensus statement from the ACC and the ADA (Brunzell et al, 2008) stated that the independent predictive power and clinical utility of fibrinogen measurement is unclear. A guideline from the National Academy of Clinical Biochemistry (Cushman et al, 2009) stated that: "There are sufficient data that fibrinogen is an independent marker of CVD risk; however. because of analytical concerns, insufficient assay standardization, and uncertainty in identifying treatment strategies, measurement is not recommended for this application."

The American Heart Association (Balagopal, et al., 2011) statement on nontraditional risk factors and biomarkers for cardiovascular disease in youth concluded: "Although studies in children suggest the presence of a prothrombotic state in obese children at an early age, the role of fibrinogen ... as potential markers of CVD risk needs to be confirmed in longitudinal studies; a cause-and-effect relationship cannot be assigned at present in children."

Guidelines from the American Association of
Clinical Endocrinologists (2012) state that fibrinogen screening in the general population is not recommended because fibrinogen levels can vary among ethnic groups. Furthermore, factors unrelated to CAD may affect fibrinogen levels and no standard measurement assay exists.

The Emerging Risk Factors Collaboration (Kaptoge, et al., 2012) analyzed individual records of 52 prospective cohort studies with 246,669 participants without a history of CVD to investigate the value of adding fibrinogen levels to conventional risk factors for the prediction of cardiovascular risk. The analysis showed that adding information of an inflammation biomarker to the standard risk factors used to predict 10-year risk of first cardiovascular event leads to a very small but statistically significant increase in the C-statistics (0.0027 for fibrinogen).

A European consensus guideline (2012) included a strong recommendation that fibrinogen should not be measured in asymptomatic low-risk individuals and high-risk patients to assess 10-year risk of CVD. The guidelines included a weak recommendation that fibrinogen may be measured as part of refined risk assessment in patients with an unusual or moderate CVD risk profile.

European guidelines (2012) identified several issues with measurement of fibrinogen for cardiovascular disease risk, including: 1) multiplicity of confounders: dependence on other classical major risk factors; 2) lack of precision: narrow diagnostic window for fibrinogen level and risk of CVD; 3) lack of specificity: similar level of risk for other non-cardiovascular causes of morbidity and mortality (e.g. other low-grade inflammatory diseases); 4) lack of dose–effect or causality relationship between changes in fibrinogen level and risk of CVD; 5) lack of specific therapeutic strategies or agents targeting circulating fibrinogen and showing reduction in CVD incidence. The guideline noted that similar observations could be made for high-sensitivity C-reactive protein. The guidelines also noted their higher cost of test compared with classical biological risk factors (e.g. blood glucose and lipids).
Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) (PLAC):  

Lipoprotein-associated phospholipase A2 (Lp-PLA2 or PLAC) testing is an enzyme immunoassay for the quantitative determination of Lp-PLA2 in plasma; used in conjunction with clinical evaluation and individual risk assessment as a suggested aid in predicting risk for coronary heart disease (CHD).

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an enzyme that can hydrolyze oxidized phospholipids to generate lysophosphatidylcholine and oxidized fatty acids, which have pro-inflammatory properties (Ballantyne et al, 2004). Based on a 510(k) premarket notification, the U.S. Food and Drug Administration has cleared for marketing the PLAC Test (diaDexus, Inc., South San Francisco, CA), an enzyme immunoassay for the quantitative determination of Lp-PLA2 in plasma.

Data regarding the association between Lp-PLA2 level and incidence of cardiovascular events are conflicting (Persson et al, 2008). Some large prospective clinical studies have found lipoprotein-associated phospholipase A2 (Lp-PLA2) to be an independent risk factor for CAD (e.g., Packard et al, 2000; Blake et al, 2001; Ballantyne et al, 2004), although another large study (Women's Health Study) found that the predictivity of Lp-PLA2 was no longer statistically significant after adjustment for other risk factors (Blake et al, 2001).

Other studies have failed to find an association between Lp-PLA2 and various cardiac disease endpoints (e.g., Kardys et al, 2006; Allison et al, 2006; Kardys et al, 2007; Rana et al, 2011; Oldgren et al, 2007). Rana et al (2011) examined the contribution of physical activity and abdominal obesity to the variation in Lp-PLA2 and other inflammatory biomarkers and incident CHD. In a prospective case-control study nested in the European Prospective Investigation into Cancer and Nutrition-Norfolk cohort, the examined the associations between circulating levels or activity of lipoprotein-associated phospholipase A2 (Lp-PLA2) and other inflammatory markers.
and CHD risk over a 10-year period among healthy men and women (45 to 79 years of age). A total of 1,002 cases who developed fatal or non-fatal CHD were matched to 1,859 controls on the basis of age, sex, and enrollment period. After adjusting for waist circumference, physical activity, smoking, diabetes, systolic blood pressure, low-density lipoprotein and high-density lipoprotein cholesterol levels, and further adjusted for hormone replacement therapy in women, Lp-PLA2 was not associated with an increased CHD risk.

A meta-analysis found Lp-PLA2 to be significantly associated with CVD (Garza et al, 2007). The researchers reported that the risk estimate appears to be relatively unaffected by adjustment for conventional CVD risk factors. The researchers reported an unadjusted odds ratio of 1.51 (95 % CI: 1.30 to 1.75) for the association between elevated Lp-PLA2 and CVD. When adjusted for traditional CVD risk factors and CRP, the odds ratio was 1.60 (95 % CI: 1.36 to 1.89). An accompanying editorial noted: "Although meta-analytic confirmation of this association is notable, clinicians must not 'jump the gun.' Important questions should be answered before Lp-PLA2 is incorporated into clinical practice, and the authors acknowledge this fully in their discussion (Steinber and Mayer, 2007). The editorialist explained that one of these questions is whether measurement of Lp-PLA2 yields additional predictive power beyond that already provided by an assessment of traditional cardiovascular risk factors and by current scoring systems such as the Framingham Risk Score. The editorialist stated that, given the weak association between Lp-PLA2 and CVD, this seems unlikely. The editorialist explained that, if a patient's baseline probability of CVD is 50 %, plotting an odds ratio of 1.60 on a Bayesian nomogram results in a posterior probability of about 59 %, a relatively small increase. "Such small changes in probability rarely translate into changes in patient management or recategorization of patients into different risk groups." The editorialist also stated that the operating characteristics of the FDA-cleared test for Lp-PLA2, the PLAC test (diaDexus Inc, San Francisco, CA), have not been adequately established (Steinberg and Mayer, 2007).
editorialist argued that decisions about the utility of a novel biomarker should not be based solely on measurements of association, such as odds ratios or relative risk. Instead, clinical decision making should be guided by the performance characteristics of the diagnostic test that measures the biomarker. The editorialist stated that test characteristics can vary significantly between patient populations. The positive and negative likelihood ratios of the PLAC test for patients at low-, intermediate-, and high-risk of various cardiovascular outcomes need to be clarified if the test is to be used in these populations. Furthermore, prospective studies need to be performed to determine whether the use of the PLAC test, or any other test of Lp-PLA2, leads to meaningful changes in patient management. "As mentioned previously, the weak association between Lp-PLA2 and CVD makes this unlikely." The editorialist also explained that the fact that Lp-PLA2 is associated with CVD does not mean it can be relied on as a surrogate marker of morbidity or mortality in clinical trials (Steinberg and Mayer, 2007). Clinical trials of drug therapy will surely track Lp-PLA2 levels, but they must also measure clinical outcomes. The editorialist also questioned whether wide-spread statin use, which has changed and grown considerably since many of the patients in previous studies were enrolled, is already offsetting the small increased risk of CVD that elevated Lp-PLA2 might confer. "This question highlights a critical goal for researchers of Lp-PLA2 drug therapy -- randomized controlled trials must be performed against background therapy that reflects current practice." The editorialist explained that, not until this work is done will we know if lowering Lp-PLA2 with targeted drug therapy is good for patients. The editorialist concluded that Lp-PLA2 should not be used for screening or risk stratification until further study. Regarding Lp-PLA2 specific drug therapy, "healthy skepticism is advised." "Responsible clinicians will resist the temptation to prescribe on the basis of pharmaceutical claims and inadequate information and wait for solid data instead."

In a prospective U.S. cohort study (Cook et al, 2006), researchers assessed whether adding measurements of
Lp-PLA2 or any of 18 other novel risk factors to traditional risk factors (age, race, sex, HDL and total cholesterol levels, systolic BP, use of anti-hypertensive agents, and smoking and diabetes status) improved prediction of incident coronary heart disease among nearly 16,000 adults (age 45 years or older). The authors found that, although Lp-PLA2 showed a statistically significant increase in predictive value compared with traditional risk factors only, this increase was not clinically important. The accompanying editorialist commented that, given that only 1 in 3 people with elevated blood pressure or cholesterol levels achieves adequate control, clinician should focus on treatment and control of traditional risk factors. The authors concluded that, for now, routine screening of Lp-PLA2 levels seems unwarranted.

An analysis of the Atherosclerosis Risk in Communities Study, which assessed the association of 19 novel risk factors with coronary heart disease in a cohort of 15,792 adults, found that measurement of Lp-PLA2 in that population added very little to the 5-year predicted risk of a coronary heart disease event based on assessment of traditional risk factors (Folsom et al, 2006). Although Lp-PLA2 was among the novel risk factors that added the most to the area under the receiver operating curve (AUC), Lp-PLA2 resulted in a very small increase in the AUC of only 0.006. The authors concluded that routine measurement of Lp-PLA2 and other novel markers is not warranted for risk assessment. The authors stated that, on the other hand, their findings reinforce the utility of major, modifiable risk factor assessment to identify individuals at risk for CHD for preventive action.

There is insufficient evidence that Lp-PLA2 is useful in reducing risk of stroke. Ballantyne et al (2005) evaluated the ability of Lp-PLA2 and C-reactive protein to predict stroke cases in a manner that is statistically independent from traditional risk factors. The authors use data from the Atherosclerosis Risk in Communities (ARIC) Study, a high-quality prospective follow-up of healthy U.S. adults with standardized risk factor measurements as well as stored blood samples that facilitated
analysis of the potential new risk predictors. As expected from prior research on stroke risk, race, hypertension, diabetes, systolic and diastolic blood pressure, and triglyceride and HDL-C levels were each individually associated with higher stroke risk. The investigators reported an association of higher Lp-PLA2 and CRP levels with increased stroke risk in statistical models adjusted for the major traditional risk factors. In the highest tertile, CRP level was associated with higher stroke risk by about 2-fold, although confidence intervals were wide. For Lp-PLA2 levels in the top tertile, with adjustment for traditional risk factors and CRP, stroke risk was higher by about 2-fold as well. Thus, the investigators found that the Lp-PLA2 level was a moderately strong stroke risk predictor, and its association with stroke in this study was statistically independent of traditional risk factors as well as the inflammatory marker CRP. In unadjusted analyses, apparently healthy middle-aged people with high levels of both CRP and Lp-PLA2 (highest tertiles of both) had a stroke risk 11-fold higher than people with low levels of both. The authors speculated that Lp-PLA2 and CRP levels may be complementary to traditional risk factors for identifying middle-aged individuals at increased risk for stroke.

The accompanying editorialists explained, however, that from the Ballantyne et al study, it is unclear how useful CRP or Lp-PLA2 level will be for improving risk prediction versus traditional risk factors alone (Greenland and O'Malley, 2005). The editorialists explained that, simply showing statistical independence is not adequate for demonstrating clinical utility for risk prediction. "Hazard ratios and p values are useful for demonstrating statistical associations, but they fail to show whether the new marker is truly capable of making a major impact on risk prediction or risk discrimination." The editorialists explained that one helpful way to determine additive utility of a new test is through the use of receiver operating characteristic (ROC) curves and AUC information. The editorialist noted that, unfortunately, Ballantyne et al did not report AUC or ROC information. However, based on statistical analytic findings reported elsewhere, individual tests with relative risks of only 2.0 to 3.0 "are simply not capable of
increasing the AUC to a clinically significant degree." The editorial concluded that "[t]o date, this search for new cardiovascular risk markers has not led to any test that can be recommended as a routine measurement beyond that of traditional risk factors."

A cohort study found no significant gain of Lp-PLA2 and minimal gains of other novel biomarkers over conventional biomarkers in predicting future cardiovascular events in a low-to-moderate risk community based population. Melander et al (2009) reported on a cohort study of 5,067 persons without cardiovascular disease from Malmö, Sweden, who attended a baseline examination between 1991 and 1994. Participants underwent measurement of Lp-PLA2, CRP, cystatin C, midregional proadrenomedullin (MR-proADM), mid-regional proatrial natriuretic peptide, and N-terminal pro-B-type natriuretic peptide (N-BNP) and underwent follow-up until 2006 using the Swedish national hospital discharge and cause-of-death registers and the Stroke in Malmö register for first cardiovascular events (MI, stroke, coronary death). During median follow-up of 12.8 years, there were 418 cardiovascular and 230 coronary events. Lp-PLA2 did not have a statistically significant relationship to cardiovascular events or coronary events, and was not retained in backwared elimination models for cardiovascular events and coronary events. Models with conventional risk factors had C statistics of 0.758 (95% CI: 0.734 to 0.781) and 0.760 (0.730 to 0.789) for cardiovascular and coronary events, respectively. Biomarkers retained in backward-elimination models were CRP and N-BNP for cardiovascular events and MR-proADM and N-BNP for coronary events, which increased the C statistic by 0.007 (p = 0.04) and 0.009 (p = 0.08), respectively. The investigators reported that the proportion of participants reclassified was modest (8% for cardiovascular risk, 5% for coronary risk). Net re-classification improvement was non-significant for cardiovascular events (0.0 %; 95% CI: -4.3 % to 4.3 %) and coronary events (4.7 %; 95% CI: -0.76 % to 10.1 %). Greater improvements were observed in analyses restricted to intermediate-risk individuals (cardiovascular events: 7.4 %; 95% CI: 0.7 % to 14.1 %; p = 0.03;
coronary events: 14.6 %; 95 % CI: 5.0 % to 24.2 %; p = 0.003). However, correct re-classification was almost entirely confined to down-classification of individuals without events rather than up-classification of those with events. In this cohort of some 5,000 participants initially free of CVD and followed almost 13 years, the novel biomarkers improved prediction scores "only minimally," resulting in the re-assignment of only 1 % of participants to a higher risk group (Melander et al, 2009).

A meta-analysis found associations of circulating Lp-PLA2 mass and activity with risk of coronary heart disease, stroke, and mortality under different circumstances (Lp-PLA(2) Studies Collaboration, 2010). The investigators conducted a meta-analysis of 39 studies to calculate risk ratios (RRs) per 1 standard deviation (SD) higher value of Lp-PLA2. The investigators found relative risks for coronary heart disease, adjusted for conventional risk factors, of 1.10 (95 % CI : 1.05 to 1.16) with Lp-PLA2 activity and 1.11 (1.07 to 1.16) with Lp-PLA2 mass. Relative risks for ischemic stroke were 1.08 (0.97 to 1.20) for LpPLA2 activity and 1.14 (1.02 to 1.27) for LpPLA2 mass. Relative risks were 1.16 (1.09 to 1.24) and 1.13 (1.05 to 1.22) for vascular mortality; and 1.10 (1.04 to 1.17) and 1.10 (1.03 to 1.18) for non-vascular mortality, respectively. Although the researchers acknowledge that further research is required in to this area, they suggest, “Randomised trials of potent reversible pharmacological inhibitors of Lp-PLA2 activity should help to establish whether modification of Lp-PLA2 can reverse vascular risk.” An accompanying editorial stated that these analyses suggest that increased Lp-PLA2 activity is associated with higher risk of coronary heart disease (Rosenson, 2010). The editorialist noted, however, that the predictive value of Lp-PLA2 activity was weaker with higher apolipoprotein B concentrations; lower concentrations of apolipoprotein B (0.85 mg/L for the mean in the lowest tertile) were associated with higher risk (1.23 [95 % CI: 1.14 to 1.33] per 1-SD change in LpPLA2 activity) than were apolipoprotein B concentrations in the higher two tertiles (1.09 [1.01 to 1.19] and 1.11 [1.03 to 1.19], respectively). The editorialist stated that future studies that evaluate the cardiovascular risks associated with Lp-PLA2
activity and/or mass should at least adjust for apolipoprotein B concentrations, and small LDL-particle concentration. The editorialist stated that these analyses are important to fully understand the contribution of increased Lp-PLA2 activity and/or mass to future risk of cardiovascular events beyond the risk obtained from quantification of LDL particles. "Clinically, the independent contribution of Lp-PLA2 concentrations or activity for risk stratification beyond the association with small LDL-particle concentration awaits the results of randomised trials that are designed to investigate whether selective and reversible inhibition of this pathway reduces cardiovascular events."

Lp-PLA2 is also being investigated for predicting outcome in acute ischemic stroke. Elkind et al (2006) reported on a population-based study of stroke risk factors in 467 patients with first ischemic stroke. The study was undertaken to determine whether levels of hs-CRP and Lp-PLA2 predict risk of stroke recurrence, other vascular events, and death. The investigators found that levels of Lp-PLA2 and hs-CRP were weakly correlated ($r = 0.09; p = 0.045$). High-sensitivity CRP, but not Lp-PLA2, was associated with stroke severity. After adjusting for age, sex, race and ethnicity, history of coronary artery disease, diabetes mellitus, hypertension, hyperlipidemia, atrial fibrillation, smoking, and hs-CRP level, compared with the lowest quartile of Lp-PLA2, those in the highest quartile had an increased risk of recurrent stroke (adjusted HR, 2.08; 95 % CI: 1.04 to 4.18) and of the combined outcome of recurrent stroke, MI, or vascular death (adjusted HR, 1.86; 95 % CI: 1.01 to 3.42).

The researchers reported that, after adjusting for confounders, hs-CRP was not associated with risk of recurrent stroke or recurrent stroke, MI, or vascular death but was associated with risk of death (adjusted HR, 2.11; 95 % CI: 1.18 to 3.75).

Whiteley et al (2009) reported on a systematic review of the evidence relating Lp-PLA2 and other blood markers and prognosis in ischemic stroke. The investigators searched Medline and EMBASE from 1966 to January 2007 for studies of blood markers in patients with ischemic stroke and an
assessment of outcome (death, disability, or handicap). The investigators found 82 studies of 41 blood markers that met inclusion criteria, including 1 study of Lp-PLA2 (citing Elkind et al, 2006). The researchers found that, although blood biomarkers might provide useful information to improve the prediction of outcome after acute ischemic stroke, the review showed that many studies were subject to bias. The researchers found that although some markers had some predictive ability, none of the studies was able to demonstrate that the biomarker added predictive power to a validated clinical model. The researchers concluded that the clinical usefulness of blood biomarkers for predicting prognosis in the setting of ischemic stroke has yet to be established.

Few studies have investigated the role of elevated Lp-PLA2 with stroke risk (Wassertheil-Smoller et al, 2008). Wassertheil-Smoller and colleagues (2008) assessed the relationship between Lp-PLA2 and the risk of incident ischemic stroke in 929 stroke patients and 935 control subjects in the Hormones and Biomarkers Predicting Stroke Study, a nested case-control study from the Women's Health Initiative Observational Study. Mean (SD) levels of Lp-PLA2 were significantly higher among case subjects (309.0 [97.1]) than control subjects (296.3 [87.3]; p < 0.01). Odds ratio for ischemic stroke for the highest quartile of Lp-PLA2, compared with lowest, controlling for multiple covariates, was 1.08 (95 % CI: 0.75 to 1.55). However, among 1,137 nonusers of hormone therapy at baseline, the corresponding odds ratio was 1.55 (95 % CI: 1.05 to 2.28), whereas there was no significant association among 737 hormone users (odds ratio: 0.70; 95 % CI: 0.42 to 1.17; p for interaction = 0.055). Moreover, among non-hormone users, women with high CRP and high Lp-PLA2 had more than twice the risk of stroke (odds ratio: 2.26; 95 % CI: 1.55 to 3.35) compared with women low levels in both biomarkers. Furthermore, different stroke cases were identified as high-risk by Lp-PLA2 rather than by CRP. The investigators concluded that Lp-PLA(2) was associated with incident ischemic stroke independently of CRP and traditional cardiovascular risk factors among non-users of hormone therapy with highest risk in those
who had both high CRP and high Lp-PLA2.

Persson et al (2008) reported on a prospective population-based study exploring the relationship between baseline Lp-PLA2 activity and mass, respectively, on levels and incidence of first CHD and ischemic stroke. Lp-PLA2 activity and mass were assessed in 5,393 (60 % women) subjects who participated in the Malmo Diet and Cancer Study cardiovascular program during 1991 to 1994. In all, 347 subjects had an event (195 CHD and 152 ischemic strokes) during the follow-up period (mean 10.6 +/- 1.7 years). In an age-, sex- and CV risk factors-adjusted Cox regression analysis, comparing top to bottom tertile of Lp-PLA2 activity, the relative risk (RR; 95 % CI): for incident CHD and ischemic stroke events were 1.48; 0.92 to 2.37 and RR: 1.94; 1.15 to 3.26, respectively. The corresponding figures for Lp-PLA2 mass were 0.95; 0.65 to 1.40 and RR: 1.92; 1.20 to 3.10. The investigators concluded that elevated levels of Lp-PLA2 activity and mass, respectively, were in this study, independently of established risk factors related to the incidence of ischemic stroke but after adjustment for lipids not significant related to incident CHD.

Nambi et al (2009) reported on a prospective case-cohort (n = 949) study in 12,762 persons in the Atherosclerosis Risk in Communities (ARIC) study, to determine whether Lp-PLA2 and hs-CRP levels improved the AUC for 5-year ischemic stroke risk. The investigators also examined how Lp-PLA2 and hs-CRP levels altered classification of individuals into low-, intermediate-, or high-risk categories compared with traditional risk factors. In a model using traditional risk factors alone, the AUC was 0.732. The addition of the biomarkers increased the AUC modestly, by 0.011 for hs-CRP alone, 0.020 for Lp-PLA2 alone, and 0.042 when hs-CRP, Lp-PLA2, and its interaction term were added. The investigators reported that, with the use of traditional risk factors to assess 5-year risk for ischemic stroke, 86 % of participants were categorized as low- risk (less than 2 %); 11 %, intermediate-risk (2 % to 5 %); and 3 %, high-risk (greater than 5 %). The addition of hs-CRP, Lp-PLA2, and their interaction to the model re-classified 4 %, 39 %, and 34 % of the
low-, intermediate- and high-risk categories, respectively. The investigators stated that, based on their analysis, the addition of both hs-CRP and Lp-PLA2 seems to satisfy the statistical requirements for a test to improve risk prediction. The investigators stated, however, that the more important question is whether the improvement conferred by the addition of the marker is clinically important and cost-effective. The investigators noted that the addition of hs-CRP and Lp-PLA2 did change risk categories in approximately 13% of the study population. "It would be ideal to validate our findings in other cohorts, conduct studies to examine if changes in therapy secondary to such a risk stratification scheme will improve ischemic stroke prevention, and examine cost-effectiveness of such a strategy."

Randomized clinical studies of statin therapy for hyperlipidemic persons have shown lower incidence of stroke in the placebo group (Armarenco and Labreuche, 2009); prospective randomized studies of statins for prevention of recurrence in stroke and TIA have shown marginal effects (Manktelow and Potter, 2009). However, it is not known whether treatment with statins would reduce stroke risk in a subset of normo-lipidemic patients for whom statin therapy would otherwise not be indicated. In addition, a number of studies have also shown that certain drugs can have an impact on Lp-PLA2 levels; these studies, however, do not demonstrate whether changes in Lp-PLA2 can improve outcomes when used as a target of treatment.

There is a lack of evidence from prospective clinical studies that incorporation of Lp-PLA2 testing in cardiovascular risk assessment improves clinical outcomes. ATPIII guidelines do not include a recommendation for Lp-PLAC testing in assessment of CAD risk. Guidelines from the American Heart Association and the American Stroke Association (Goldstein et al, 2006) on primary prevention of ischemic stroke state: "No recommendations about Lp-PLA2 modification can be made because of an absence of outcome studies showing clinical benefit with reduction in its blood levels." A consensus
statement from the American College of Cardiology and the American Diabetes Association on management of patients with cardiometabolic risk makes no mention of Lp-PLA2 (Bruzell et al., 2008). The American Association of Clinical Chemistry (AACC, 2009) has stated that Lp-PLA2 is not widely available, and, "while the findings from recent studies support the potential usefulness of Lp-PLA2 in CHD and ischemic stroke risk assessment, its ultimate clinical utility has yet to be established." Canadian Cardiovascular Society guidelines (Genest, et al., 2009) do not recommend Lp-PLA2 for screening for heart disease risk. The American College of Cardiology and the American Heart Association (2010) examined Lp-PLA2 and concluded that it might be reasonable for assessment in intermediate-risk asymptomatic adults. This was a class IIb recommendation, indicating that the recommendation's usefulness/efficacy is less well established.

European consensus guidelines (2012) state that the magnitude of Lp-PLA2's effect on risk remains modest at the level of the general population; study limitations or bias are present. The guidelines state that LpPLA2 remains a "second-line" marker for CVD risk estimation. The guidelines suggest that LpPLA2 may be measured as part of a refined risk assessment in patients at high risk of a recurrent acute atherothrombotic event. This is a class IIb recommendation, indicating that the recommendation's usefulness/efficacy is less well established.

The American Stroke Association and the American Heart Association (Goldstein, et al., 2011) also rendered a class IIb recommendation for the use of Lp-PLA2. "Measurement of inflammatory markers such as hs-CRP or Lp-PLA2 in patients without CVD may be considered to identify patients who may be at increased risk of stroke, although their effectiveness (ie, usefulness in routine clinical practice) is not well established."

Guidelines from the American College of Clinical Endocrinology (2012) has a grade 2B recommendation to use highly sensitive CRP to stratify CVD risk in patients with a standard risk assessment that is borderline, or in those with an LDL-C
concentration less than 130 mg/dL, and to measure Lp-PLA2 when it is necessary to further stratify a patient’s CVD risk.


An ad-hoc panel of Lp-PLA2 investigators recommended consensus guidelines for Lp-PLA2 use in clinical practice (Davidson et al, 2008). The panel recommended Lp-PLA2 testing as an adjunct to traditional risk factors in determining the target goal for lipid treatment in correlation with absolute risk. The panel did not recommend Lp-PLA2 testing as a screening tool for low-risk patients. Commenting on these guidelines, Ali and Madjid (2009) stated that it is to be noted that these recommendations are based on consensus, and that more evidence is needed to determine the exact clinical approach for use of Lp-PLA2 as a screening test and as part of a treatment regimen.

Bertoia et al (2013) examined the prospective association between oxidation-specific biomarkers, primarily oxidized phospholipids (OxPL) on apolipoprotein B-100-containing lipoproteins (OxPL/apoB) and lipoprotein (a) [Lp(a)], and risk of PAD. These researchers examined, as secondary analyses, indirect measures of oxidized lipoproteins, including autoantibodies to malondialdehyde-modified low-density lipoprotein (MDA-LDL) and apolipoprotein B-100 immune complexes (ApoB-IC). The study population included 2 parallel nested case-control studies of 143 men within the Health Professionals Follow-up Study (1994 to 2008) and 144 women within the Nurses' Health Study (1990 to 2010) with incident confirmed cases of clinically significant PAD, matched 1:3 to control subjects. Levels of OxPL/apoB were positively
associated with risk of PAD in men and women: pooled relative risk: 1.37, 95% CI: 1.19 to 1.58 for each 1-SD increase after adjusting age, smoking, fasting status, month of blood draw, lipids, BMI, and other cardiovascular disease risk factors. Lipoprotein (a) was similarly associated with risk of PAD (pooled adjusted relative risk: 1.36; 95% CI: 1.18 to 1.57 for each 1-SD increase). Autoantibodies to MDA-LDL and ApoB-IC were not consistently associated with risk of PAD. The authors concluded that OxPL/apoB were positively associated with risk of PAD in men and women. The major lipoprotein carrier of OxPL, Lp(a), was also associated with risk of PAD, reinforcing the key role of OxPL in the pathophysiology of atherosclerosis mediated by Lp(a).

The main drawbacks of this study included: (i) because the NHS and HPFS studies contain predominantly white subjects, it is unclear if these findings can be generalized to minority populations, some of whom are at increased risk for PAD, (ii) it is possible that some control subjects have undiagnosed PAD, and (iii) these finding alone cannot definitely separate OxPL and Lp(a) as individual determinants of PAD, given their inherent biological inter-relationship. The authors stated that “Future research should continue to explore the mechanisms that link oxidation to risk of PAD and test whether modifiable risk factors, potentially including novel therapies that reduce levels of OxPL, might prevent the development of atherosclerotic diseases such as PAD”.

The Emerging Risk Factors Collaboration (Di Angelantonio, et al., 2012) found, in a study of individuals without known CVD, the addition of information on Lp-PLA2 to risk scores containing total cholesterol and HDL-C led to slight improvement in CVD prediction. Individual The investigators estimated that for 100,000 adults aged 40 years or older, 15,436 would be initially classified at intermediate risk using conventional risk factors alone. Additional testing with Lp-PLA2 would reclassify 2.7% of people to a 20% or higher predicted CVD risk category and, therefore, in need of statin treatment under Adult Treatment Panel III guidelines.
Carotid Intima-Media Thickness:

Carotid intima media thickness (IMT) testing measures the thickness of the inner two layers of the wall of the carotid artery. The intima is the innermost layer and the media is the middle layer of the arterial wall. An ultrasound image is used to detect carotid IMT which can purportedly diagnose early stages of atherosclerosis, before symptoms occur and assess for drug efficacy. It is thought that a thickening of the carotid intima media confirms the likelihood of atherosclerosis of other arteries, including the coronary and carotid arteries. This led to the theory that carotid IMT could be used to identify persons at high risk for cardiovascular and cerebrovascular disease. Examples of US Food and Drug Administration (FDA) approved IMT devices include ArterioVision and CardioHealth Station.

Carotid ultrasonography measurement of the intimal medial thickness of the carotid arteries has been used to assess the atherosclerotic plaque burden. Increased carotid intimal medial thickness has been correlated with a gradual, graded increase in the risk of future cardiovascular events, but the magnitude of the relationship lessened when traditional risk factors were taken into account (Chambless et al, 1997; Hodis et al, 1998; O'Leary et al, 1999; Simons et al, 1999; Touboul et al, 2000; Bots et al, 2007; Lorenz et al, 2007).

ATPIII reports that the extent of carotid atherosclerosis correlates positively with the severity of coronary atherosclerosis, and that some studies have shown that severity of intimal medial thickness independently correlates with risk for major coronary events. ATPIII states, however, that the predictive power of carotid medial intima thickness for persons without multiple risk factors has not been determined in prospective studies. ATPIII concluded that “its expense, lack of availability, and difficulties with standardization preclude a current recommendation for its use in routine risk assessment for the purpose of modifying intensity of LDL-lowering therapy.”

A consensus statement from the ADA and the ACC observed
that measurements of carotid intima media thickness, as well as measurement of coronary calcification and ankle-brachial index, can detect the presence of so-called subclinical vascular disease, and that patients with documented subclinical atherosclerosis are at increased CVD risk and may be considered candidates for more aggressive therapy. The consensus statement concluded, however, that it is unclear whether such tests improve prediction or clinical decision making in patients with cardiometabolic risk (Brunzell et al, 2008).

The U.S. Preventive Services Task Force (USPSTF, 2009) stated that there is insufficient evidence to recommend the use of carotid intima-media thickness to screen asymptomatic individuals with no history of CHD to prevent CHD events.

American Association of Clinical Endocrinology (2012) guidelines state that carotid intima media thickness measurements should not be performed routinely, but may be used in certain clinical situations as adjuncts to standard CVD risk factors in an attempt to refine risk stratification and the need for more aggressive preventive strategies. This is a grade 4 recommendation, based upon opinion (D level evidence).

An American Heart Association guideline on cardiovascular disease in women (Mosca, et al., 2011) stated: "Although recent evidence suggests that using imaging modalities such as coronary calcium scoring and carotid ultrasound to demonstrate the presence of advanced atherosclerosis has the greatest utility for reclassifying risk in those (including women) predicted to be at intermediate risk on the basis of short-term risk equations such as the Framingham risk score, their value in improving clinical outcomes has not been established."

An assessment prepared for the Agency for Healthcare Research and Quality (Helfand, et al., 2009) concluded that "carotid intima media thickness ... probably provide[s] independent information about coronary heart disease risk, but data about their prevalence and impact when added to
Framingham risk score in intermediate-risk individuals are limited.

Guidelines from the Canadian Cardiovascular Society (Anderson, et al., 2013) noted that a recent metaanalysis found that carotid intima media measurements added only little to risk reclassification after adjustment for conventional risk factors.

van den Oord et al. (2013) conducted a systematic review and meta-analysis of the evidence on the association of carotid intima media thickness with future cardiovascular events and the additional value of carotid intima media thickness to traditional cardiovascular risk prediction models. The association of carotid intima media thicknes with future cardiovascular events and the additional value of carotid intima media thickness were calculated using random effects analysis. The literature search yielded 1196 articles of which 15 articles provided sufficient data for the meta-analysis. A 1 standard deviation increase in carotid intima media thickness was predictive for myocardial infarction (HR 1.26, 95% CI 1.20-1.31) and for stroke (HR 1.31, 95% CI 1.26-1.36). A 0.1 mm increase in carotid intima media thickness was predictive for myocardial infarction (HR 1.15, 95% CI 1.12-1.18) and for stroke (HR 1.17, 95% CI 1.15-1.21). The overall performance of risk prediction models did not significantly increase after addition of carotid intima media thickness data. The areas under the curve increased from 0.726 to 0.729 (p = 0.8). The authors concluded that carotid intima media thickness as measured by B-mode ultrasound is associated with future cardiovascular events. However, the addition of carotid intima media thickness to traditional cardiovascular risk prediction models does not lead to a statistical significantly increase in performance of those models.

Den Ruijter (2012) conducted a metaanalysis to determine whether common carotid intima media thickness has added value in 10-year risk prediction of first-time myocardial infarctions or strokes, above that of the Framingham Risk Score.
The authors identified relevant studies through literature searches of databases (PubMed from 1950 to June 2012 and EMBASE from 1980 to June 2012) and expert opinion. The included studies if participants were drawn from the general population, common carotid intima media thickness was measured at baseline, and individuals were followed up for first-time myocardial infarction or stroke. The authors combined individual data into one data set and they performed an individual participant data meta-analysis on individuals without existing cardiovascular disease. The authors included 14 population-based cohorts contributing data for 45,828 individuals. During a median follow-up of 11 years, 4007 first-time myocardial infarctions or strokes occurred. The authors first refitted the risk factors of the Framingham Risk Score and then extended the model with common carotid intima media thickness measurements to estimate the absolute 10-year risks to develop a first-time myocardial infarction or stroke in both models. The C statistic of both models was similar (0.757; 95% CI, 0.749-0.764; and 0.759; 95% CI, 0.752-0.766). The authors found that the net reclassification improvement with the addition of common carotid intima media thickness was small (0.8%; 95% CI, 0.1%-1.6%). In those at intermediate risk, the net reclassification improvement was 3.6% in all individuals (95% CI, 2.7%-4.6%) with no differences between men and women. The authors concluded that the addition of common carotid intima media thickness measurements to the Framingham Risk Score was associated with small improvement in 10-year risk prediction of first-time myocardial infarction or stroke, but this improvement is unlikely to be of clinical importance.

Guidelines from the American College of Cardiology and the American Heart Association (Goff, et al., 2014) state that "routine measurement of CIMT is not recommended in clinical practice for risk assessment for a first ASCVD event."

Carotid Ultrasound Screening:

The United States Preventive Services Task Force (USPSTF, 2014)
recommends against screening for asymptomatic carotid artery stenosis in the general population of adults without a history of transient ischemic attack, stroke, or other neurologic signs and symptoms. This is a D recommendation, meaning that the USPSTF recommends against this service because there is moderate or high certainty that the service has no net benefit or that the harms outweigh the benefits. The USPSTF observed that the most feasible screening test for carotid artery stenosis (defined as 60% to 99% stenosis) is ultrasonography. The USPSTF stated that, although adequate evidence indicates that this test has high sensitivity and specificity, in practice, ultrasonography yields many false-positive results in the general population, which has a low prevalence of carotid artery stenosis (approximately 0.5% to 1%). The USPSTF also found that there are no externally validated, reliable tools that can determine who is at increased risk for carotid artery stenosis or for stroke when carotid artery stenosis is present. The USPSTF found that all screening strategies, including ultrasonography with or without confirmatory tests (digital subtraction or magnetic resonance angiography), have imperfect sensitivity and could lead to unnecessary surgery and result in serious harms, including death, stroke, and myocardial infarction. The USPSTF concluded with moderate certainty that the harms of screening for asymptomatic carotid artery stenosis outweigh the benefits.

Measurement of Arterial Elasticity:

Arterial elasticity has been shown to decrease with aging and with vascular disease. A number of studies have demonstrated loss of arterial elasticity in persons with CAD, heart failure, hypertension and diabetes.

Arterial stiffness, measured as aortic pulse wave velocity between the carotid and femoral arteries, appears to be a predictor of cardiovascular events (Mattace-Raso et al, 2006; Willum-Hansen et al, 2006). In the Rotterdam Study, the adjusted relative risk for coronary disease or stroke in the 2nd and 3rd tertiles was 1.72 and 2.45 compared to the lowest
Hypertension Diagnostics, Inc. (HDI, Eagan, MN) has developed a method of analyzing blood pressure waveforms to noninvasively measure the elasticity (compliance) of arteries and arterioles. The HDI CVProfilor and the HDI PulseWave graphs the blood pressure waveform ("pulse contour analysis") and calculates the elasticity (flexibility) of large and small arteries and arterioles. The CVProfilor obtains blood pressure and waveform data by use of a blood pressure cuff placed on the left upper-arm and a piezoelectric-based, direct contact, acoustical transducer placed over the right radial artery near the wrist. A computer performs a pulse contour analysis of blood pressure waveform data, and generates a report which includes a large artery elasticity index (a measure of capacitative compliance) and a small artery elasticity index (a measurement of oscillatory or reflective compliance). The CVProfilor also provides measurements of standard blood pressure values (systolic, diastolic and mean arterial pressure), heart rate, body surface area (BSA) and BMI. Arterial elasticity has been investigated as an early marker of vascular disease in patients without standard risk factors for CVD. Several studies have examined the impact of various factors on arterial elasticity, and have examined the question of whether arterial elasticity is an independent risk factor for cardiovascular disease. However, there is inadequate evidence from prospective clinical studies demonstrating that non-invasive measurements of arterial elasticity using the CVProfilor alters patient management and improves clinical outcomes. Current guidelines from leading medical professional organizations do not include a recommendation for use of pulse waveform analysis in cardiovascular disease risk assessment.

In a clinical trial, Woodman et al (2005) reported that large and small artery compliance, and stroke volume/pulse pressure (measured by HDI/PulseWave CR-2000), and systemic arterial...
compliance show poor agreement with central pulse wave velocity, an established measure of central arterial stiffness.

**Interleukin 6 -174 g/c Promoter Polymorphism:**

Inflammation plays an important role in the pathogenesis of atherosclerosis. Interleukin 6 (IL-6) has many inflammatory functions, and the IL-6 -174 g/c promoter polymorphism appears to influence IL-6 levels. Previous findings on the relation between this polymorphism and risk of CVD are inconsistent. Sie and colleagues (2006) examined this polymorphism in relation to risk of CHD in a population-based study and meta-analysis. Subjects (n = 6,434) of the Rotterdam Study were genotyped. Analyses on the relation between genotype and CHD were performed using Cox proportional hazards tests, and the association between genotype and plasma levels of IL-6 and CRP was investigated. All of the analyses were adjusted for age, sex, and common cardiovascular risk factors. A meta-analysis was performed, using a random effects model. No association between genotype and risk of CHD was observed. The polymorphism was not associated with IL-6 levels, but the C-allele was associated with higher CRP levels (p < 0.01). This meta-analysis did not show a significant association between the genotype and risk of CHD. The authors concluded that the polymorphism is not a suitable genetic marker for increased risk of CHD in persons aged 55 years or older.

In men, plasma interleukin-6 (IL-6) concentrations have been shown to be predictive of a future myocardial infarction (Ridker et al, 2000; Woods, et al, 2000), but its contribution to risk of MI is attenuated significantly when other risk factors are taken into account (Pai et al, 2004).

**Myeloperoxidase (MPO):**

Myeloperoxidase (MPO) is an enzyme found in white blood cells that is purportedly linked to inflammation and cardiovascular disease. Higher levels of the leukocyte enzyme
myeloperoxidase (MPO), which is secreted during acute inflammation and promotes oxidation of lipoproteins, are associated with the presence of coronary disease (Zheng et al, 2001; Zheng et al, 2004) and may be predictive of acute coronary syndrome in patients with chest pain (Brennan et al, 2003). Stefanescu et al (2008) found that patients with stable CAD had increased CVD risk if plasma MPO levels were elevated and a small study demonstrated that MPO deficiency may protect against CVD (Kutter and Devaquet, 2000). Furthermore, among patients with chronic systolic heart failure (HF), elevated plasma MPO levels have been associated with an increased likelihood of more advanced HF and may be predictive of a higher rate of adverse clinical outcomes (Tang et al, 2007).

Although elevated plasma MPO concentration may be associated with a more advanced CVD risk profile, plasma MPO does not predict mortality independent of other CVD risk factors in patients with stable CAD. There is a lack of scientific evidence regarding how measurements of MPO would affect management of individuals at risk for or patients with CHD. Large randomized controlled studies are needed to ascertain the clinical value of MPO in the management of CHD.

**Apolipoprotein A-1**

Apolipoprotein A1 (Apo A1) is the major protein constituent of HDL cholesterol and a relatively abundant plasma protein. Apo-A1 is instrumental in promoting the transfer of cholesterol into the liver where it is metabolized and then excreted from the body via the intestine. Although most guidelines recommend cardiovascular risk assessment based on LDL, measurement of Apo A1 has not been established as a clinically useful test at this time. It has not been proven useful in determining therapy for patients with CAD or dyslipemia.

Apolipoproteins are measured in routine clinical laboratories with the use of immunonephelometric or immunoturbidimetric assays. Importantly, international standards have been developed for apolipoprotein B100 (apoB) and A-1 (Mora,
ApoB reflects the number of potentially atherogenic lipoprotein particles because each particle of very-low-density lipoprotein (VLDL), β-VLDL, intermediate-density lipoprotein (IDL), LDL, and lipoprotein(a) particle carries on its surface 1 apoB100 protein. Most of plasma apoB is found in LDL particles. HDL particles do not carry apoB but instead carry apolipoprotein A-1 (apoA-1). However, apoA-1 does not correspond directly to the concentration of HDL particles in the 1-to-1 fashion seen for apoB100 and LDL particles because an HDL particle may carry >1 apoA-1 protein (Mora, 2009).

While Ridker et al (2005) found that Apo A1 predicts cardiovascular disease, it has no more predictive value than more readily available markers, such as the non-HDL cholesterol level and the ratio of total to HDL cholesterol. In a secondary analysis of a large prospective cohort study involving 15,632 healthy women in the Women's Health Study, investigators assessed the value of several markers. Subjects were followed for at least 10 years, during which time 464 had first cardiovascular events (MI, ischemic stroke, coronary revascularization, or death). After adjustment for age, smoking status, blood pressure, diabetes, and BMI, the hazard ratios for a first cardiovascular event in the most extreme quintiles for each marker (compared with the most favorable quintiles) were as follows: LDL cholesterol level, 1.62; apolipoprotein A-I level, 1.75; total cholesterol level, 2.08; HDL cholesterol level, 2.32; apolipoprotein B level, 2.50; non-HDL cholesterol level, 2.51; CRP level, 2.98. For lipid ratios, the hazard ratios were: apo B:apo A-I, 3.01; LDL:HDL cholesterol, 3.18; apo B:HDL cholesterol, 3.56; total:HDL cholesterol, 3.81.

A case control study found that the ratio of apolipoprotein B to apolipoprotein A-I was associated with coronary artery disease but added little to existing measures of risk assessment (van der Steeg et al, 2007). United Kingdom researchers evaluated whether the ratio of apolipoprotein B to apolipoprotein A-I was associated with CAD among 869 adults with CAD and 1,511 controls matched for age, sex, and time of enrollment. The highest quartile of the apolipoprotein ratio was significantly
associated with fatal and non-fatal CAD (odds ratio, 1.85) in analyses adjusted for cardiovascular risk factors (sex, diabetes, BMI, smoking, systolic blood pressure, CRP levels, and LDL and HDL cholesterol levels). The ratio also was associated with CAD (odds ratio [OR], 1.77) in analyses adjusted for the Framingham risk score (a well-established algorithm for combining risk factors to predict CAD). However, the total/HDL cholesterol ratio and the apolipoprotein ratio categorized cases and controls similarly. In addition, the proportion of people with CAD who were predicted to have higher risk for CAD was similar when both ratios were used and when the apolipoprotein ratio was added to the Framingham risk score. An editorialist commented that "risk factor proliferation puts patients and clinicians at risk for confusion" (Berkwits and Guallar, 2007).

A report from the Framingham Offspring Study, a large, population-based, cohort study, found that apo A-1 ratio has little clinical utility in predicting incident coronary heart disease, and that measuring total cholesterol and HDL appears to suffice to determine heart disease risk (Ingelsson et al, 2007). More than 3,300 middle-aged, white participants in the Framingham Offspring Study without CVD were followed for a median of 15 years. A total of 291 first CHD events occurred, 198 of them in men. In men, elevations in non-HDL cholesterol, apo B, total cholesterol:HDL ratio, LDL:HDL ratio, and apo B:apo A-1 ratio were all significantly associated with increased CHD risk to a similar degree. Elevated apo A-1 and HDL were likewise associated with reduced CHD risk. Women had results similar to those in men except that decreased apo A-1 was not significantly associated with incident CHD. In sex-specific analyses, elevated LDL and total cholesterol were not significantly associated with increased CHD risk in either men or women, perhaps owing to the lack of statistical power of these substudies. In men, total cholesterol:HDL and apo B:apo A-1 ratios both improved re-classification of 10-year risk for CHD; however, the difference between the two was not significant. In women, neither lipid ratio improved CHD risk re-classification.
A large observational study reported that apolipoproteins were better than HDL and LDL in cardiac disease risk assessment (McQueen et al, 2008). In the INTERHEART study, 12,461 patients with acute MI from the world's major regions and ethnic groups were compared with 14,637 age- and sex-matched controls to assess the contributions of various cardiovascular risk factors. Investigators obtained nonfasting blood samples from 9,345 cases and 12,120 controls and measured cholesterol fractions and apolipoproteins to determine their respective predictive values. The investigators found that ratios were stronger predictors of MI than were individual components, and apolipoproteins were better predictors than their cholesterol counterparts. The Apo B/Apo A1 ratio was the strongest predictor, with a population-attributable risk of 54%, compared with risks of 37% for LDL/HDL and 32% for total cholesterol/HDL. A 1-standard-deviation increase in Apo B/Apo A1 was associated with an odds ratio of 1.59 for MI, compared with 1.17 for an equivalent increase in total cholesterol/HDL. The results were similar for both sexes and across all ethnic groups and ages. The authors argued that Apo B and Apo A1 should be used in clinical practice worldwide for cardiovascular risk assessment. A commentator noted, however, that no prospective evidence indicates that such a change would improve clinical outcomes (Soloway, 2008).

A meta-analysis found no relationship between apo A1 and apo B and stroke risk (Emerging Risk Factors Collaboration, 2009). An individual-patient meta-analysis, aimed at providing clear estimates of the vascular risks associated with lipid levels, included 68 prospective studies with data on 302,430 people without vascular disease at baseline; of these, 32 studies provided data on ischemic stroke outcomes in more than 173,000 people. Non-HDL cholesterol level was modestly associated with ischemic stroke risk, but triglyceride and HDL cholesterol levels were not associated with either ischemic or hemorrhagic stroke risk. Both non-HDL and HDL cholesterol levels were associated with cardiac risk. Measurement of apo B and apo A-I did not add predictive value.
The NCEP report concludes that Apo A1 is not appropriate for routine cardiovascular risk screening. An ACC/ADA consensus statement (Brunzell et al, 2008) concluded that measurements of Apo A1 appears to provide little clinical value beyond measurements of HDL cholesterol.

A European consensus statement (2012) explains Apo A1 is the major apoprotein of HDL. The consensus stated that "it is beyond doubt that the apoB:apoA1 ratio is one of the strongest risk markers." The guidelines note, however, that it is still not established whether this variable should be used as a treatment goal. "As the measurement of apolipoproteins is not available to all physicians in Europe, is more costly than currently used lipid variables, and does not add more information, its use is not as yet generally recommended."

**Peripheral Arterial Tonometry**

Endothelium plays an important role in the maintenance of vascular homeostasis. Nitric oxide (NO) is the key mediator of endothelial function; it is a potent vasodilator, it inhibits platelet aggregation, vascular smooth muscle cell migration and proliferation, and monocytes adhesion. Cardiovascular risk factors promote development of endothelial dysfunction, characterized by impairment of endothelium-dependent vasodilation (EDV) and by pro-coagulant/pro-inflammatory endothelial activities. The assessment of EDV is a common parameter for testing endothelial function. Endothelium-dependent vasodilation in the coronary arteries is angiographically evaluated by measurement of the vessel response to endothelial agonists, such as acetylcholine (gold standard). A non-invasive technique for the detection of EDV employs the ultrasound evaluation of flow-mediated dilation (FMD) of the brachial artery following reactive hyperemia. A close relation between FMD and coronary vasomotor response to acetylcholine has been reported. Endothelial dysfunction in the coronary circulation may precede development of angiographically evident coronary atherosclerosis; endothelial dysfunction has been also associated with a higher prevalence
of CAD and resulted predictive of future cardiovascular events; recently, it has been associated with a higher risk of re-stenosis after coronary stent implantation. Endothelial dysfunction is actually considered a reversible phenomenon; drug therapies with angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers, statins, anti-oxidants agents have shown a beneficial effect on endothelial function (Patti et al, 2005).

Peripheral arterial tonometry (PAT) has been proposed as a non-invasive method to measure endothelial dysfunction and potentially identify patients with early-stage CAD. Endothelial dysfunction is measured by the PAT signal that is obtained using the Endo-PAT2000 device (Itamar Medical) and proprietary software. The test involves the measurement of blood flow in the fingertips following compression of the upper arm with an inflatable cuff. The Endo-PA2000 was cleared by the FDA through the 510(k) process in November 2003. It is indicated for use as a diagnostic aid in the detection of coronary artery endothelial dysfunction (positive or negative) using a reactive hyperemia procedure. The device is not intended for use as a screening test in the general patient population. However, there is currently insufficient evidence to support the use of PAT in assessing CAD risk.

Kuvin et al (2007) assessed endothelial function in 2 peripheral vascular beds before and during reactive hyperemia in an outpatient clinic setting. The brachial artery was imaged with a portable ultrasound device and changes in vessel diameter were expressed as "% FMD". Pulse wave amplitude of the finger was detected by PAT and PAT hyperemia was defined as the maximal plethysmographic recording compared to baseline. A total of 60 individuals (43 men) were enrolled with an average age 53 +/- 2 years (mean +/- SE). The 31 individuals with more than 2 cardiac risk factors (CRF) had lower FMD (7.0 +/- 1.1 %) and PAT hyperemia (2.1 +/- 0.9) compared to the 29 persons with 0 to 2 CRF (FMD 11.3 +/- 0.8 %, PAT hyperemia 2.4 +/- 0.1; p < 0.05 for both). The 32 individuals with CAD had lower FMD (6.8 +/- 1.1 %) and PAT hyperemia (2.0 +/- 0.1)
compared to the 28 individuals without CAD (FMD 11.5 +/- 0.8 
%, PAT hyperemia 2.4 +/- 0.1; p < 0.05 for both). Thus, 
peripheral vascular endothelial function testing in the 
ambulatory setting correlates with the extent of CAD risk and 
the presence or absence of CAD. The authors concluded that 
these data suggested that peripheral vascular endothelial 
function testing is feasible in ambulatory patients, and this is an 
important next step in bringing this technology to clinical 
applicability.

Ghiadoni et al (2008) stated that the endothelium plays a key 
role in the maintenance of vascular homeostasis. A 
dysfunctional endothelium is an early marker of the 
development of atherosclerotic changes and can also 
contribute to cardiovascular events. Vascular reactivity tests 
represent the most widely used methods in the clinical 
assessment of endothelial function and in the last 2 decades, 
several methodologies were developed to study it non‐ 
invasively in the peripheral macro‐circulation (conduit 
arteries) and micro‐circulation (resistance arteries and 
arterioles). These investigators reviewed the most relevant 
available non‐invasive techniques in the research on endothelial 
function, their 
advantages and limitations. Flow mediated dilation of 
the brachial artery by ultrasounds is the most widely used 
vascular test to ascertain endothelium‐ dependent vasodilation. 
Other approaches include measurement of micro‐circulatory 
reactive hyperemia by fore‐arm venous plethysmography or 
digital pulse amplitude tonometry, response to beta‐2 agonist 
by applanation tonometry or digital photo‐plethysmography 
and several test by skin laser Doppler. It appears that FMD is 
the most reproducible test when an appropriate and accurate 
methodology is applied. Systemic markers proposed as 
measures of NO biology, inflammatory cytokines, adhesion 
molecules, or markers of endothelial damage and repair have 
only a very limited role as a result of biological and assay 
availability and variability, these factors currently have a limited 
role in the assessment of individual patients. The optimal 
methodology for investigating the multi‐faceted aspects of
endothelial dysfunction is still under debate. Thus, no available test to assess endothelial function has sufficient sensitivity and specificity to be used yet in clinical practice. Only the growing concordant results from different reproducible and reliable non-invasive methods examining endothelial function with different stimuli will support and strengthen experimental findings, thus providing conclusive answers in this area of research.

Chemla and associates (2008) reviewed recent advances in the non-invasive assessment of arterial pressure (indirect methods) in the field of critical care. Automated oscillo-metric measurements underestimate intra-arterial systolic blood pressure. Digital photo-plethysmography has led to conflicting results, although the obtained respiratory pulse pressure variation correlates with the fluid-challenge-induced changes in stroke volume. The pulse oximetry photo-plethysmographic signal recorded at the digital or ear level may be useful in monitoring respiratory arterial pressure variations, although technical improvements and clarifications are needed. Arterial tonometry is increasingly used in the cardiovascular field to reconstruct central aortic pressure. A recent study has shown that radial artery tonometry is feasible in hemodynamically stable patients and that peripheral pulse pressure reflects the combined influences of arterial stiffness and stroke volume, especially in elderly patients. The limitations of this technique include the potential bias related to the use of a generalized transfer function and the difficulty in obtaining reliable recordings in hemodynamically unstable patients. The authors concluded that intra-arterial blood pressure must be preferred over non-invasive blood pressure recordings when critical decisions are required. In hemodynamically stable patients, valuable information may be obtained by using non-invasive techniques, amongst which arterial tonometry seems promising.

Burg et al (2009) stated that myocardial ischemia provoked by emotional stress (MSI) in patients with stable CAD predicts major adverse cardiac events. These researchers tested an
easily administered, non-invasive technology to identify vulnerability to mental stress ischemia. Patients with documented CAD (n = 68) underwent single photon emission CT myocardial perfusion imaging concurrent with pulse wave amplitude assessment by PAT during a mental stress protocol of sequential rest and anger stress periods. Heart rate and blood pressure were assessed, and blood was drawn for catecholamine assay, during rest and stress. Myocardial ischemia provoked by emotional stress was defined by the presence of a new perfusion defect during anger stress (n = 26) and the ratio of stress to rest PAT response was calculated. Patients with MSI had a significantly lower PAT ratio than those without MSI (0.76 +/- 0.04 versus 0.91 +/- 0.05, p = 0.03). An ROC curve for optimum sensitivity/specificity of PAT ratio as an index of MSI produced a sensitivity of 0.62 and a specificity of 0.63. Among patients taking ACE inhibitors, the sensitivity and specificity of the test increased to 0.86 and 0.73, respectively; 90 % of patients without MSI were correctly identified. The authors concluded that PAT in concert with ACE inhibition may provide a useful approach to assess risk for MSI. They stated that future studies should help determine how best to utilize this approach for risk assessment in the clinical setting.

B-Type Natriuretic Peptides:

Brain natriuretic peptide (BNP) is a hormone produced in the body that, when elevated, may be an indication of congestive heart failure (CHF). BNP testing may be used to detect this hormone and aid in the diagnosis of CHF. N-terminal pro-BNP (NT-proBNP) is the precursor molecule for BNP. BNP or NT-proBNP testing has been proposed for the determination of CVD risk and may be included in CVD risk testing panels.

In a systematic review and meta-analysis on B-type natriuretic peptides (BNP) and cardiovascular risk, Di Angelantonio and colleagues (2009) stated that measurement of BNP concentration or its precursor (N-terminal fragment [NT-proBNP]) is recommended in patients with symptoms of left ventricular dysfunction and in other settings, but the
relevance of these peptides to CVD in general populations or in patients with stable vascular disease is uncertain. These investigators collated data from 40 long-term prospective studies involving a total of 87,474 participants and 10,625 incident CVD outcomes. In a comparison of individuals in the top-third with those in the bottom-third of baseline values of natriuretic peptides, the combined RR, adjusted for several conventional risk factors, was 2.82 (95 % CI: 2.40 to 3.33) for CVD. Analysis of the 6 studies with at least 250 CVD outcomes (which should be less prone to selective reporting than are smaller studies) yielded an adjusted RR of 1.94 (95 % CI: 1.57 to 2.39). Risk ratios were broadly similar with BNP or NT-proBNP (RR, 2.89 [95 % CI: 1.91 to 4.38] and 2.82 [95 % CI: 2.35 to 3.38], respectively) and by different baseline vascular risk (RR, 2.68 [95 % CI: 2.07 to 3.47] in approximately general populations; RR, 3.35 [95 % CI: 2.38 to 4.72] in people with elevated vascular risk factors; RR, 2.60 [95 % CI, 1.99 to 3.38] in patients with stable CVD). Assay of BNP or NT-proBNP in addition to measurement of conventional CVD risk factors yielded generally modest improvements in risk discrimination. The authors concluded that available prospective studies indicate strong associations between circulating concentration of natriuretic peptides and CVD risk under a range of different circumstances. They stated that further investigation is warranted, particularly in large general population studies, to clarify any predictive utility of these markers and to better control for publication bias.

Melander and co-workers (2009) assessed the utility of contemporary biomarkers for predicting cardiovascular risk when added to conventional risk factors. A total of 5,067 participants (mean age of 58 years; 60 % women) without CVD were included in this study. Participants underwent measurement of CRP, cystatin C, Lp-PLA2, mid-regional pro-adrenomedullin (MR-proADM), mid-regional pro-atrial natriuretic peptide, and N-terminal pro-B-type natriuretic peptide (N-BNP) and underwent follow-up using the Swedish national hospital discharge and cause-of-death registers and the Stroke in Malmo register for first cardiovascular events
Main outcome measures were incident cardiovascular and coronary events. During median follow-up of 12.8 years, there were 418 cardiovascular and 230 coronary events. Models with conventional risk factors had C statistics of 0.758 (95% CI: 0.734 to 0.781) and 0.760 (0.730 to 0.789) for cardiovascular and coronary events, respectively. Biomarkers retained in backward-elimination models were CRP and N-BNP for cardiovascular events and MR-proADM and N-BNP for coronary events, which increased the C statistic by 0.007 (p = 0.04) and 0.009 (p = 0.08), respectively.

The proportion of participants re-classified was modest (8% for cardiovascular risk, 5% for coronary risk). Net re-classification improvement was non-significant for cardiovascular events (0.0%; 95% CI: -4.3% to 4.3%) and coronary events (4.7%; 95% CI: -0.76% to 10.1%). Greater improvements were observed in analyses restricted to intermediate-risk individuals (cardiovascular events: 7.4%; 95% CI: 0.7% to 14.1%; p = 0.03; coronary events: 14.6%; 95% CI: 5.0% to 24.2%; p = 0.003). However, correct re-classification was almost entirely confined to down-classification of individuals without events rather than up-classification of those with events. The authors concluded that selected biomarkers may be used to predict future cardiovascular events, but the gains over conventional risk factors are minimal. Risk classification improved in intermediate-risk individuals, mainly through the identification of those unlikely to develop events. They stated that "[t]hese data do not exclude a future role for circulating biomarkers as adjuncts to conventional risk factors, nor do they minimize the potential for biomarkers to provide insight into underlying mechanisms of diseases. Several biomarkers studied did lead to shifts in predictive accuracy that were at least statistically significant. The challenge will be to find new cardiovascular biomarkers that alone or in combination with existing biomarkers can bring about improvements in risk assessment that are not just statistically but clinically significant as well".

Commenting on this study, Schwenk (2009) concluded that this study "shows that several markers that are associated with CAD and other cardiovascular diseases in high-risk populations do
not provide much incremental predictive value over known
demographic and clinical risk factors in low-to-moderate risk
community-based populations. For now, more-precise
personalized approaches to risk stratification and subsequent
prevention of cardiovascular disease are not available."

An assessment by the National Academy of Clinical
Biochemistry (Christenson et al, 2009) stated
that measurement of B-type natriuretic peptide (BNP) or N-
terminal proBNP (NT-proBNP) concentrations for CVD risk
assessment in the primary prevention setting is unwarranted.
Similarly, guidelines from the American College of Cardiology
and the American Heart Association (2010) do not recommend
measurement of natriuretic peptides for CVD risk assessment in
asymptomatic adults

Using specific immunoassay and tandem mass spectrometry,
Siriwarden et al (2010) showed that a fragment derived from
the signal peptide of B-type natriuretic peptide (BNPsp) not
only is detectable in cytosolic extracts of explant human heart
tissue but also is secreted from the heart into the circulation of
healthy individuals. Furthermore, plasma levels of BNPsp in
patients with documented acute ST-elevation myocardial
infarction (n = 25) rise to peak values (about 3 times higher
than the 99th percentile of the normal range) significantly
earlier than the currently used biomarkers myoglobin, creatine
kinase-MB, and troponin. Preliminary receiver-operating
characteristic curve analysis comparing BNPsp concentrations in
ST-elevation MI patients and other patient groups was positive
(AUC = 0.97; p < 0.001), suggesting that further, more rigorous
studies in heterogeneous chest pain patient cohorts are
warranted. The authors concluded that these findings
demonstrated for the first time that BNPsp exists as a distinct
entity in the human circulation and could serve as a new class
circulating biomarker with the potential to accelerate the
clinical diagnosis of cardiac ischemia and myocardial infarction.

In an editorial that accompanied the afore-mentioned article,
Ichiki and Burnett (2010) stated that the study was small (n =
If the current findings are confirmed, then BNPsp17-26 may markedly increase the armamentarium of cardiac biomarkers for myocardial ischemia and injury. They noted that further studies are needed.

Guidelines from the Royal Australian College of General Practitioners (2012) stated that the evidence for screening for heart failure using BNP is mixed despite its sensitivity and prognostic significance. The guidelines stated that BNP may be useful in excluding the condition in suspected heart failure.

**Mid-Regional Pro-Atrial Natriuretic Peptide:**

The rapid and reliable estimation of prognosis in acute ischemic stroke is pivotal to optimize clinical care. Mid-regional pro-atrial natriuretic peptide (MR-proANP), a recently described, stable fragment of the ANP precursor hormone, may be useful in this setting. In a prospective observational study, Katan and colleagues (2010) examined the prognostic value of MR-proANP in patients with acute ischemic stroke. These researchers measured MR-proANP on admission in plasma of 362 consecutive patients presenting with acute ischemic stroke. The prognostic value of MR-proANP to predict mortality within 90 days and functional outcome (defined as a modified Rankin Scale of less than or equal to 2 or greater than or equal to 3) was evaluated and compared with the National Institutes of Health Stroke Scale (NIHSS) score. The discriminatory accuracy, calculated with the AUC of the receiver operating characteristics curve, of MR-proANP to predict death was comparable to the NIHSS (AUC: 0.86 [95 % CI: 0.82 to 0.90] and 0.85 [95 % CI: 0.81 to 0.89; p = 0.7]). Combined, the accuracy significantly improved (0.92 [95 % CI: 0.88 to 0.96; p < 0.01]). The AUC of MR-proANP to predict functional outcome was 0.70 (95 % CI: 0.65 to 0.75), similar to the NIHSS (0.75 [95 % CI: 0.70 to 0.80]; p = 0.16). The prognostic value of MR-proANP for both outcomes was independent of the NIHSS. Higher MR-proANP concentrations were found in stroke of cardioembolic etiology. The authors concluded that MR-proANP is a prognostic marker in the acute phase of stroke,
improving the discriminatory value of the NIHSS, independently predicting post-stroke mortality and functional outcome.

In an editorial that accompanied the paper by Katan et al, Granger and Laskowitz (2010) stated that the current study was performed at a single center with only 44 deaths, and the results need to be validated in an independent study. A number of important questions remain. does this biomarker change predicted risk enough to alter recommended therapy? Does use of the biomarker result in improved care and clinical outcomes? And is it cost-effective?


**Measurement of Long-Chain Omega-3 Fatty Acids in Red Blood Cell Membranes:**

Long-chain omega-3 fatty acids are a family of unsaturated fatty acids that have in common a carbon-carbon double bond in the third bond from the methyl end of the fatty acid. Omega-3 fatty acids cannot be manufactured by the body and are obtained from foods such as fish (eg, salmon, halibut), certain plants and nut oils. Serum long-chain omega-3 fatty acids have been suggested as a cardiac risk factor for sudden cardiac death.

Higher palmitic and lower long-chain omega-3 fatty acids (e.g., alpha-linolenic, eicosapentaenoic and docosahexaenoic acids) in serum are correlated with higher incidence of CHD in middle-aged men at high risk for CVD (Simon et al, 1995). Improvements in plasma fatty acids and vitamins E and C were the only factors found related to improvements in life expectancy and 70% lowering of heart disease in a study population (Renaud et al, 1995).

Harris (2004) stated that consumption of between 450 and
1,000 mg/day of long-chain omega-3 fatty acids (fish or fish oil) is recommended for those without and with known CHD, respectively. Based on animal and isolated cell studies, these fatty acids were presumed to have anti-arrhythmic effects. It has been proposed that red blood cell (RBC) fatty acids composition, which is an index of long-term intake of eicosapentaenoic plus docosahexaenoic acids, can be considered a new, modifiable, and clinically relevant risk factor for death from CHD.

However, there is a lack of scientific evidence regarding how measurements of RBC omega-3 fatty acids composition would affect management of individuals at risk for or patients with CHD. Large RCTs are needed to ascertain the clinical value of RBC omega-3 fatty acids composition in the management of CHD.

**Total Cholesterol Content in Erythrocyte Membranes:**

Plaque rupture in acute coronary syndrome (ACS) depends at least partly on the volume of the necrotic lipid core. Histopathological studies have suggested that cholesterol transported by erythrocytes and deposited into the necrotic core of atheromatous plaques contributes to lipid core growth. Tziakas and colleagues (2007) hypothesized that cholesterol content is increased in the circulating erythrocytes of patients with ACS and may be a marker of clinical instability. Thus, these researchers investigated if cholesterol content differs in erythrocyte membranes of patients presenting with ACS compared to patients with chronic stable angina (CSA). Consecutive angina patients were prospectively assessed; 120 had CSA (83 men, age of 64 +/- 11 years) and 92 ACS (67 men, age of 66 +/- 11 years). Total cholesterol content in erythrocyte membranes (CEM) was measured using an enzymatic assay, and protein content was assessed by the Bradford method. The CEM (median and inter-quartile range) was higher (p < 0.001) in ACS patients (184 microg/mg; range of 130.4 to 260.4 microg/mg) compared with CSA patients (81.1 microg/mg; range of 53.9 to 109.1 microg/mg) (analysis of co-variance).
Total plasma cholesterol concentrations did not correlate with CEM levels ($r = -0.046$, $p = 0.628$). The authors concluded that thes findings showed for the first time that CEM is significantly higher in patients with ACS compared with CSA patients. They suggested a potential role of CEM as a marker of atheromatous plaque growth and vulnerability. The authors stated that further studies are needed to elucidate the role of CEM as both a marker of plaque instability and a pathogenic mechanism of rapid CAD progression. In an editorial that accompanied the afore-mention article, Arbustini (2009) noted that "although widely investigated either as total cholesterol content or phospholipid/cholesterol ratio, CEM did not find relevant clinical applications".

Tziakas and associates (2009) evaluated the effect of statin therapy on CEM levels (a novel marker of CAD instability) during a 1-year follow-up in CAD patients. A total of 212 consecutive eligible patients (158 men, mean age of 62 +/- 10 years) undergoing diagnostic coronary angiography for the assessment of angina pectoris were assessed. The study population comprised of 84 CSA patients and 128 ACS patients. All study participants were commenced on statin treatment in equipotent doses and were followed for up to 1 year (at 1, 3, 6 and 12 months). Repeated measurements analysis of variance after appropriate adjustment showed a significant decrease ($p < 0.001$) in CEM content during follow-up. Levels of CEM were decreasing at each time point (1 month: 100 microg/mg; 95% CI: 94.3 to 105.6, 3 months: 78.1 microg/mg; 95% CI: 73.2 to 83, 6 months: 67.2 microg/mg; 95% CI: 63.1 to 71.2; 1 year: 45.3 microg/mg; 95% CI: 42.2 to 48.3) compared to admission (112.1 microg/mg; 95% CI: 105.9 to 118.3) and to all previous measurements. The authors concluded that these findings showed that the use of statins is associated with a reduction in CEM, an emerging marker of clinical instability and plaque vulnerability in CAD patients. The pleiotropic effects of statins at the cell membrane level represent a promising novel direction for research in CAD.

9p21 and Other Genetic Tests:
Some labs offer a variety of genetic tests to attempt to predict risk of cardiovascular diseases. These tests include the following:

- 4q25 genotype testing (eg, 4q25-AF Risk Genotype Test and Cardio IQ 4q25-AF Risk Genotype Test) has been proposed to identify individuals at risk of atrial fibrillation (AF) and cardioembolic (CE) stroke.
- 9p21 genotype testing has been proposed to predict risk of early myocardial infarction (MI), abdominal aortic aneurysm (AAA) and MI/coronary heart disease (CHD).
- LPA Intron-25 genotype testing (eg, Cardio IQ LPA Intron-25 Genotype Test and LPA-Intron 25 Genotype Test) has been proposed to predict risk of CHD.

Single-nucleotide polymorphism (SNP)-based testing (eg, Cardiac Healthy Weight DNA Insight, Heart Health Genetic Test) analyzes a variety of genes to identify risk factors purportedly associated with heart conditions including AF and coronary artery disease (CAD).

Labs may also offer genetic or SNP-based tests that can reportedly promote and influence general health and wellness by analyzing genes associated with response to diet, metabolism and exercise. An example of this type of test is Healthy Woman DNA Insight.

Additionally, genetic studies to determine risk of hypercoagulation or thrombosis have been proposed to determine risk for cardiovascular disease (CVD). Panels typically include factor II (ie, F2 gene), factor V (ie, F5 gene) or plasminogen activator inhibitor (PAI-1).

The Evaluation of Genomic Applications in Practice and Prevention Working Group (EWG, 2010) noted that prevention of CVD is a public health priority. Improvements in outcomes associated with genomic profiling may have important impacts. Traditional risk factors such as those used in the Framingham Risk Scores have an advantage in clinical screening and risk
assessment strategies because they measure the actual targets for therapy (e.g., lipid levels and blood pressure). To add value, genomic testing may lead to better outcomes than those achievable by assessment and treatment of traditional risk factors alone. Some issues important for clinical utility remain unknown, such as the biological mechanism underlying the most convincing marker's (9p21) association with CVD; the level of risk that changes intervention; whether long-term disease outcomes will improve; how individuals ordering direct to consumer tests will understand/respond to test results and interact with the health care system; and whether direct to consumer testing will motivate behavior change or amplify potential harms. It has been suggested that an improvement in CVD risk classification (adjusting intermediate-risk of CVD into high- or low-risk categories) might lead to management changes (e.g., earlier initiation or higher rates of medical interventions, or targeted recommendations for behavioral change) that improve CVD outcomes. In the absence of direct evidence to support this possibility, the EWG sought indirect evidence aimed at documenting the extent to which genomic profiling alters CVD risk estimation, alone and in combination with traditional risk factors, and the extent to which risk re-classification improves health outcomes. Assay-related evidence on available genomic profiling tests was deemed inadequate. However, based on existing technologies that have been or may be used and on data from 2 of the companies performing such testing, the analytic sensitivity and specificity of tests for individual gene variants might be at least satisfactory. A total of 29 gene candidates were evaluated, with 58 different gene variant/disease associations. Evidence on clinical validity was rated inadequate for 34 of these associations (59 %) and adequate for 23 (40 %). Inadequate grades were based on limited evidence, poor replication, existence of possible biases, or combinations of these factors. For heart disease (25 combined associations) and stroke (13 combined associations), profiling provided areas under the receiver operator characteristics curve of 66 % and 57 %, respectively. Only the association of 9p21 variants with heart disease had convincing evidence of a per-allele odds ratio of
between 1.2 and 1.3; this was the highest effect size for any variant/disease combination with at least adequate evidence. Although the 9p21 association seems to be independent of traditional risk factors, there is adequate evidence that the improvement in risk prediction is, at best, small. Clinical utility was not formally evaluated in any of the studies reported to date, including for 9p21. As a result, no evidence was available on the balance of benefits and harms. Also, there was no direct evidence available to assess the health benefits and harms of adding these markers to traditional risk factors (e.g., Framingham Risk Score). However, the estimated additional benefit from adding genomic markers to traditional risk factors was found to be negligible. In summary, the EWG found insufficient evidence to recommend testing for the 9p21 genetic variant or 57 other variants in 28 genes to assess risk for CVD in the general population, specifically heart disease and stroke. The EWG found that the magnitude of net health benefit from use of any of these tests alone or in combination is negligible. The EWG discourages clinical use unless further evidence supports improved clinical outcomes. Based on the available evidence, the overall certainty of net health benefit is deemed "low."

Guidelines from the American College of Cardiology and the American Heart Association (2010) state that genotype testing for CHD risk assessment in asymptomatic adults is not recommended.

Guidelines from the Canadian Cardiovascular Society (2009) state that genetic testing for severe lipoprotein disorders is available in a few highly specialized centres. The guidelines state, however, that a molecular genetic diagnosis is not necessary for the majority of patients with severe dyslipidemia; the biochemical and clinical data usually suffice to make a diagnosis. As a research tool, however, the molecular study of extreme lipoprotein disorders has provided considerable scientific insight including the identification of potential future therapeutic targets.
European consensus guidelines (2012) make a strong recommendation that DNA-based tests for common genetic polymorphisms do not presently add significantly to diagnosis, risk prediction, or patient management and cannot be recommended. The guidelines also make a strong recommendation that the added value of genotyping, as an alternative or in addition to phenotyping, for a better management of risk and early prevention in relatives, cannot be recommended.

European guidelines (2012) note that "a number of genetic polymorphisms (sequence variants that occur at a frequency >1%) appear to be associated with statistically significant effects on risk at the population level. Because of the polygenic and polyfactorial determinants of the most common CVDs, the impact of any single polymorphism remains rather modest. Genetic testing can identify variants associated with increased risk to individual CVD risk factors, CHD, or stroke. Commercial testing was recently made available to predict an individual's genetic risk, including direct-to-consumer testing. The clinical benefits of commercial testing have not yet been demonstrated."

A review of genomics of cardiovascular disease published in the New England Journal of Medicine (O'Donnell & Nabel, 2011) concluded: "Genetic risk prediction is at an early stage, and insufficient evidence exists at present to warrant the use of a genetic risk score on the basis of SNPs identified through genomic approaches. Additional research is needed to prospectively assess the utility of genetic risk scores in the prediction of cardiovascular disease, such as myocardial infarction and coronary artery disease, before clinical use."

A statement from the American Heart Association (Ashley, et al., 2012) on genetics and cardiovascular disease states: "Although robust [genome wide association studies] evidence exists linking common variants to complex CVD, studies are not yet available to inform the clinical benefit of providing such genetic information to patients."
Guidelines from the American Stroke Association and the American Heart Association (Goldstein, et al., 2011) state: 'Although commercially available tests exist for the 9p21 and 4q25 risk loci, studies have yet to show that knowledge of genotypes at these loci leads to an improvement in risk prediction or measurable and cost-effective improvements in patient care.'

Guidelines from the Royal Australian College of General Practitioners (2012) state that there is limited evidence on the balance of benefits and harms of genomic profiling generally, as well as ethical issues and uncertain utility.

**Kinesin-Like Protein 6 (KLP6)**

Kinesin family member 6 (KIF6) genotype testing has been proposed to be used to aid in the assessment of individuals being considered for statin medication therapy.

Genome-wide association studies have revealed connections between a number of common single nucleotide polymorphisms (SNPs) and cardiovascular diseases. Kinesin-like protein 6 is a protein involved in intra-cellular transport expressed in many tissues and cell types. A SNP located in the kinesin-like family 6 (KIF6) gene substitutes a thymidine (T) for a cytosine (C), resulting in substitution of arginine for tryptophan at amino acid 719 (p.Trp719Arg) of the KIF6 protein.

Prospective studies have suggested that carriers of the 719Arg allele in KIF6 are at increased risk of clinical coronary artery disease compared with noncarriers. There have been claims that non-carriers of the KIF6 719Arg variant receive little benefit from statin therapy. Screening for this genetic variant is now being used to influence statin use. Celera Corporation (Alameda, CA) has marketed its KIF6 Trp719Arg variant assay (Statincheck) to cardiologists and primary care physicians. However, a recent study found that statin therapy significantly reduced the incidence of coronary and other major vascular events to a similar extent, irrespective of KIF6 genotype (Hopewell et al, 2011). Investigators sought to test the effects
of the KIF6 Trp719Arg polymorphism (rs20455) on vascular risk and response to statin therapy in 18,348 participants from the Heart Protection Study. Study subjects received 40 mg simvastatin daily for 4 to 6 weeks before being randomly allocated 40 mg simvastatin daily or placebo for 5 years. Major coronary event was pre-defined as coronary death or non-fatal MI, and major vascular event was pre-defined as major coronary event plus re-vascularization or stroke. The investigators found that the KIF6 genotype was not significantly associated, among placebo-allocated participants, with the risks of incident major vascular events, major coronary events, re-vascularizations, or strokes. Overall, 40 mg simvastatin daily produced a 42 % reduction in low-density lipoprotein cholesterol, which did not differ significantly by KIF6 719Arg carrier status (p = 0.51). Proportional reductions in the risk of major vascular events with statin therapy were similar (interaction p = 0.70) and highly significant across KIF6 genotypes: 23% (95 % CI: 16 % to 29 %; p = 5.3 × 10^{-10}) in carriers (Arg/Arg or Trp/Arg), and 24 % (95 % CI: 17% to 31%; p = 4.6 × 10^{-9}) in non-carriers (Trp/Trp). A similar lack of interaction was observed for major coronary events, re-vascularizations, and strokes considered separately. The authors concluded that the use of KIF6 genotyping to guide statin therapy is not warranted as statin therapy significantly reduced the incidence of coronary and other major vascular events to a similar extent, irrespective of KIF6 genotype.

Previously, a large replication study found that the KIF6 Trp719Arg polymorphism was not associated with the risk of clinical CAD (Assimes et al, 2010). Investigators sought to replicate the association between the kinesin-like protein 6 (KIF6) Trp719Arg polymorphism (rs20455), and clinical CAD. The KIF6 Trp719Arg polymorphism (rs20455) was genotyped in 19 case-control studies of non-fatal CAD either as part of a genome-wide association study or in a formal attempt to replicate the initial positive reports. A total of 17,000 cases and 39,369 controls of European descent as well as a modest number of South Asians, African Americans, Hispanics, East
Asians, and admixed cases and controls were successfully genotyped. None of the 19 studies demonstrated an increased risk of CAD in carriers of the 719Arg allele compared with non-carriers. Regression analyses and fixed-effects meta-analyses ruled out with high-degree of confidence an increase of 2% in the risk of CAD among European 719Arg carriers. Investigators also observed no increase in the risk of CAD among 719Arg carriers in the subset of Europeans with early-onset disease (younger than 50 years of age for men and younger than 60 years of age for women) compared with similarly aged controls as well as all non-European subgroups. The investigators concluded that the KIF6 Trp719Arg polymorphism was not associated with the risk of clinical CAD in this large replication study. Accompanying editorialists commented on the lack of a plausible mechanism for the relationship between KIF6, statins, and heart disease (Topol and Damani, 2010). The editorialists stated that "the KIF6 story should serve as a valuable reminder of the potential pitfalls present in prematurely adopting a genomic test without sufficient evidence."

**Osteoprotegerin:**

Venuraju and colleagues (2010) stated that osteoprotegerin (OPG) is a glycoprotein that acts as a decoy receptor for receptor activator of nuclear factor kappaB ligand (RANKL) and tumor necrosis factor (TNF)-related apoptosis-inducing ligand. The OPG/RANKL/receptor activator of nuclear factor kappaB axis plays an important regulatory role in the skeletal, immune, and vascular systems. The protective role of OPG, in animal models, against vascular calcification has not been replicated in human trials; moreover, increased OPG levels have been consistently associated with the incidence and prevalence of CAD. There seems to be some dichotomy in the role of OPG, RANKL, and TNF-related apoptosis-inducing ligand in atherosclerosis and plaque stability. These researchers integrated the findings from some of the important studies and try to draw conclusions with a view to gaining some insight into the complex interactions of the OPG/RANKL/receptor activator
of nuclear factor kappaB axis and TNF-related apoptosis-inducing ligand in the pathophysiology of atherosclerosis. The authors concluded that while the clinical prognostic utility of OPG appears to be awhile away yet, it does hold a great deal of promise in helping clinicians risk stratify patients with CVD more accurately.

**The CardioVision MS-2000:**

The CardioVision MS-2000 is an electronic BP device that calculates an "arterial stiffness index" ("ASI") related to how fast the BP falls as the air pressure is released from the BP cuff. Sharma et al (2005) noted that several methods may be used to determine arterial stiffness. One method obtains an ASI from the vascular dynamics of oscillometric-derived brachial artery pressure. These researchers determined the test-retest repeatability of the CardioVision MS-2000. A total of 47 healthy hospital employees had 5 consecutive measurements of ASI measured after a 5- to 10-min period of rest and then repeated after an average of 146.8 days. Their mean age was 37 years and 71% were women. The meanASI was 39.6 +/- 9.7 and 37.2 +/- 10.5 mm Hg x10 (p = 0.22) for the 1st and 2nd time period, respectively. These researchers computed an intra-class correlation coefficient of 0.31 and 0.33 for the 1st and 2nd time periods, which is the measure of consistency or agreement of ASI values within cases. The intra-class correlation coefficient for systolic BP, diastolic BP, heart rate and ASI were 0.68 (p = 0.0001), 0.70 (p = 0.0001), 0.35 (p = 0.02) and 0.25 (p = 0.08), respectively. The authors concluded that the results of this study suggested poor test-retest repeatability if consecutive measurements are used. The intra-class correlation coefficient, however, could be improved by eliminating the highest and lowest value from a set of measurements.

According to Barrett (2010), "arterial stiffness measurements can identify some people who are at risk for cardiovascular disease. However, they are not as reliable or cost-effective as standard blood pressure and cholesterol screenings."
Non-traditional risk markers have been shown to have statistically significant independent associations with incident CHD, but Folsom et al (2006) found that they do very little to improve the predictive value of traditional risk-factor assessment. Using a series of case-cohort studies, the prospective Atherosclerosis Risk in Communities (ARIC) Study assessed the association of 19 novel risk markers with incident CHD in 15,792 adults followed up since 1987 to 1989 (Folsom et al, 2006). Novel markers included measures of inflammation (CRP, LpPLA2, interleukin 6, D-dimer), endothelial function (intra-cellular adhesion molecule-1), fibrin formation (plasminogen activator inhibitor-1, tissue inhibitor of metalloproteinase-1, soluble thrombomodulin, E-selectin), fibrinolysis (matrix metalloproteinase-1, plasminogen, tissue plasminogen activator), B vitamins (leptin, homocysteine, folate, vitamin B6), and antibodies to infectious agents (Chlamydia IgG positivity, cytomegalovirus antibody, herpes simplex virus-1 antibody). Change in the AUC was used to assess the additional contribution of novel risk markers to CHD prediction beyond that of traditional risk factors. The investigators found that the basic risk factor model, which included traditional risk factors (age, race, sex, total and high-density lipoprotein cholesterol levels, systolic blood pressure, anti-hypertensive medication use, smoking status, and diabetes), predicted coronary heart disease well, as evidenced by an AUC of approximately 0.8. The other risk factors did not add significantly to the AUC. Among the novel risk factors, the greatest contribution to AUC was CRP, with an increase in AUC of 0.003. The authors concluded that routine measurement of these novel markers is not warranted for risk assessment. These findings also reinforced the utility of major, modifiable risk factor assessment to identify individuals at risk for CHD for preventive action. The accompanying editorialists explained that these novel markers should not be used for basic risk factor assessment because they do not meaningfully reduce mis-classification by traditional risk scoring (Lloyd-Jones and Tian, 2006).
Lloyd-Jones and Tian (2006) explained that statistical association of a novel marker with CVD that is "independent" of traditional risk factors is necessary but far from sufficient to demonstrate utility in the prediction of CVD. Rather, predictive utility requires demonstration of improvement in test characteristics, predictive values, AUCs (or C statistics), or likelihood ratios at given cutoff values when a novel marker is added to the existing risk score. They explained that, from a decision-making point of view, the "ultimate" measure of a novel screening test is its ability to reclassify individuals. In other words, a new marker is useful only when it corrects a substantial portion of mis-classification by the old test (the existing risk score).

The AHA's scientific statement on "Nontraditional risk factors and biomarkers for cardiovascular disease: Mechanistic, research, and clinical considerations for youth" (Balagopal et al, 2011) listed novel biomarkers for CVD in children including adiponectin, leptin, peroxisome proliferator-activated receptor, retinol binding protein 4, and resistin. Balagopal et al (2011) also stated that "Numerous other products are secreted by adipocytes .... Other discoveries include visfatin, touted to play an important role in regulation of glycemic homeostasis, and apelin, the function of which appears to be related to regulation of nutritional intake. The role of these and other adipokines in CVD and T2DM remains unclear". Regarding cytokines, the AHA stated: "Further research verifying these findings [cytokins such as IL-6 and TNF-α] and better evaluating non-CRP inflammatory processes as they relate to CVD and CVD risk factors in childhood will be valuable."

In a prospective population-based study, Kavousi et al (2012) evaluated if newer risk markers for CHD risk prediction and stratification improve Framingham risk score (FRS) predictions. A total of 5,933 asymptomatic, community-dwelling participants (mean age of 69.1 years [SD, 8.5]) were included in this analysis. Traditional CHD risk factors used in the FRS (age, sex, systolic blood pressure, treatment of hypertension, total and high-density lipoprotein cholesterol levels, smoking, and
diabetes) and newer CHD risk factors (N-terminal fragment of prohormone B-type natriuretic peptide levels, von Willebrand factor antigen levels, fibrinogen levels, chronic kidney disease, leukocyte count, CRP levels, Hcy levels, uric acid levels, coronary artery calcium [CAC] scores, carotid intima-media thickness, peripheral arterial disease, and pulse wave velocity). Adding CAC scores to the FRS improved the accuracy of risk predictions (c-statistic increase, 0.05 [95% CI: 0.02 to 0.06]; net re-classification index, 19.3% overall [39.3% in those at intermediate-risk, by FRS]). Levels of N-terminal fragment of prohormone B-type natriuretic peptide also improved risk predictions but to a lesser extent (c-statistic increase, 0.02 [CI: 0.01 to 0.04]; net re-classification index, 7.6% overall [33.0% in those at intermediate-risk, by FRS]). Improvements in predictions with other newer markers were marginal. The authors concluded that among 12 CHD risk markers, improvements in FRS predictions were most statistically and clinically significant with the addition of CAC scores. Moreover, they stated that further investigation is needed to assess whether risk refinements using CAC scores lead to a meaningful change in clinical outcome.

Guidelines from the American Association of Clinical Endocrinology (2012) do not recommend the routine measurement of plasminogen activator inhibitor 1 and uric acid because the benefit in doing so is unclear.

Zampetaki et al (2012) noted that circulating miRNAs are emerging as potential biomarkers. These researchers examined the association between baseline levels of microRNAs (miRNAs) and incident myocardial infarction (MI) in the Bruneck cohort and determined their cellular origin. A total of 19 candidate miRNAs were quantified by real-time polymerase chain reactions in 820 participants. In multi-variable Cox regression analysis, 3 miRNAs were consistently and significantly related to incident MI: miR-126 showed a positive association (multi-variable HR: 2.69 [95% CI: 1.45 to 5.01], p = 0.002), whereas miR-223 and miR-197 were inversely associated with disease risk (multi-variable HR: 0.47 [95% CI: 0.29 to 0.75], p = 0.002,
and 0.56 [95% CI: 0.32 to 0.96], p = 0.036). To determine their cellular origin, healthy volunteers underwent limb ischemia-reperfusion generated by thigh-cuff inflation, and plasma miRNA changes were analyzed at baseline, 10 mins, 1 hr, 5 hrs, 2 days, and 7 days. Computational analysis using the temporal clustering by affinity propagation algorithm identified 6 distinct miRNA clusters. One cluster included all miRNAs associated with the risk of future MI. It was characterized by early (1 hr) and sustained activation (7 days) post-ischemia-reperfusion injury and consisted of miRNAs predominantly expressed in platelets. The authors concluded that in subjects with subsequent MI, differential co-expression patterns of circulating miRNAs occur around endothelium-enriched miR-126, with platelets being a major contributor to this miRNA signature. The authors stated that these findings await confirmation in independent cohorts. Also, causality cannot be inferred from associations of biomarkers in population studies. Furthermore, they stated that future studies will need to address whether endothelial and platelet miRNAs can serve as novel biomarkers for clinical-decision making.

In an editorial that accompanied the afore-mention study, Engelhardt (2012) stated that the limitations of the study included: (i) despite a considerable size of the study cohort, the total number of incident cases of MI is relatively low, (ii) only a pre-defined panel of miRNAs was analyzed in all participants. This panel was initially chosen based on pattern analysis of miRNA expression in a smaller sub-population and then applied to the entire cohort. Important candidates (e.g., miRNAs) that only show deregulation in cases of incident MI may have been over-looked, (iii) the present findings await confirmation in an independent cohort before one may proceed to make use of these miRNAs as useful tools to enhance stratification for MI.

Jaguszewski et al (2014) noted that Takotsubo cardiomyopathy (TTC) remains a potentially life-threatening disease, which is clinically indistinguishable from acute MI. Currently, no established biomarkers are available for the early diagnosis of TTC and differentiation from MI. MicroRNAs (miRNAs/miRs)
emerge as promising sensitive and specific biomarkers for cardiovascular disease. Thus, these investigators sought to identify circulating miRNAs suitable for diagnosis of acute TTC and for distinguishing TTC from acute MI. After miRNA profiling, 8 miRNAs were selected for verification by real-time quantitative reverse transcription polymerase chain reaction (PCR) in patients with TTC (n = 36), ST-segment elevation acute MI (STEMI, n = 27), and healthy controls (n = 28). They quantitatively confirmed up-regulation of miR-16 and miR-26a in patients with TTC compared with healthy subjects (both, p < 0.001), and up-regulation of miR-16, miR-26a, and let-7f compared with STEMI patients (p < 0.0001, p < 0.05, and p < 0.05, respectively). Consistent with previous publications, cardiac specific miR-1 and miR-133a were up-regulated in STEMI patients compared with healthy controls (both, p < 0.0001). Moreover, miR-133a was substantially increased in patients with STEMI compared with TTC (p < 0.05). A unique signature comprising miR-1, miR-16, miR-26a, and miR-133a differentiated TTC from healthy subjects [area under the curve (AUC) 0.835, 95 % CI: 0.733 to 0.937, p < 0.0001] and from STEMI patients (AUC 0.881, 95 % CI: 0.793 to 0.968, p < 0.0001). This signature yielded a sensitivity of 74.19 % and a specificity of 78.57 % for TTC versus healthy subjects, and a sensitivity of 96.77 % and a specificity of 70.37 % for TTC versus STEMI patients. Additionally, these researchers noticed a decrease of the endothelin-1 (ET-1)-regulating miRNA-125a-5p in parallel with a robust increase of ET-1 plasma levels in TTC compared with healthy subjects (p < 0.05). The authors concluded that the present study for the first time described a signature of four circulating miRNAs as a robust biomarker to distinguish TTC from STEMI patients. They stated that the significant up-regulation of these stress- and depression-related miRNAs suggested a close connection of TTC with neuropsychiatric disorders. Moreover, decreased levels of miRNA125a-5p as well as increased plasma levels of its target ET-1 are in line with the microvascular spasm hypothesis of the TTC pathomechanism.

Roncarati et al (2014) stated that myocardial miRNAs modulate
processes such as cardiomyocyte (CM) hypertrophy, excitation-contraction coupling, and apoptosis; non-CM-specific miRNAs regulate myocardial vascularization and fibrosis. Recently, the possibility that circulating miRNAs may be biomarkers of cardiovascular disease has been raised. These researchers examined if microRNAs (miRNAs) involved in myocardial remodeling were differentially expressed in the blood of hypertrophic cardiomyopathy (HCM) patients, and whether circulating miRNAs correlated with the degree of left ventricular hypertrophy and fibrosis. A total of 41 HCM patients were characterized with conventional transthoracic echocardiography and cardiac magnetic resonance. Peripheral plasma levels of 21 miRNAs were assessed by quantitative real-time PCR and were compared with levels in a control group of 41 age- and sex-matched blood donors. Twelve miRNAs (miR-27a, -199a-5p, -26a, -145, -133a, -143, -199a-3p, -126-3p, -29a, -155, -30a, and -21) were significantly increased in HCM plasma. However, only 3 miRNAs (miR-199a-5p, -27a, and -29a) correlated with hypertrophy; more importantly, only miR-29a correlated also with fibrosis. The authors concluded that these findings suggested that cardiac re-modeling associated with HCM determined a significant release of miRNAs into the bloodstream: the circulating levels of both cardiac- and non-cardiac-specific miRNAs were significantly increased in the plasma of HCM patients. However, correlation with left ventricular hypertrophy parameters held true for only a few miRNAs (i.e., miR-199a-5p, miR-27a, and miR-29a), whereas only miR-29a was significantly associated with both hypertrophy and fibrosis, identifying it as a potential biomarker for myocardial re-modeling assessment in HCM.

UpToDate reviews on “Screening for coronary heart disease” (Yanowitz, 2013), “Screening for coronary heart disease in patients with diabetes mellitus” (Bax et al, 2013), “Management of proximal left anterior descending coronary artery disease” (Bell and Bittl, 2013), and “Management of left main coronary artery disease” (Cutlip, 2013) do not mention the use of coronary artery reactivity testing.
Itabe et al (2011) stated that accumulating evidence indicates that oxidized low-density lipoprotein (OxLDL) is a useful marker for cardiovascular disease. The uptake of OxLDL by scavenger receptors leads to the accumulation of cholesterol within the foam cells of atherosclerotic lesions. OxLDL has many stimulatory effects on vascular cells, and the presence of OxLDL in circulating blood has been established. According to the classical hypothesis, OxLDL accumulates in the atherosclerotic lesions over a long duration, leading to advanced lesions. However, recent studies on time-course changes of OxLDL in-vivo raised a possibility that OxLDL can be transferred between the lesions and the circulation. These investigators discussed the in-vivo dynamics of OxLDL. The authors concluded that recent studies have suggested the plasma OxLDL concentrations may change under pre-pathological and post-pathological conditions. OxLDL may be transferred between tissues and plasma and does not merely accumulate in the lesions but is equilibrated between the tissues and circulation. OxLDL can be formed in various sites in addition to the tissue of the vessel wall. The liver is the major organ for the clearance of OxLDL from circulation. However, many unknowns remain to be elucidated regarding the metabolic fate of OxLDL in the liver. A recent study pointed out that stabilins may have an important role in the recognition and clearance of OxLDL and MM-LDL from circulation. The receptors working in the liver may be different from those of cells in vessel wall tissues. They stated that further studies are needed to understand the in-vivo behavior of OxLDL and elucidate the contributions of OxLDL and oxidative stress to the mechanism of atherogenesis.

An UpToDate reviews on "Overview of the risk equivalents and established risk factors for cardiovascular disease" (Wilson, 2013) and "Screening for coronary heart disease" (Yanowitz, 2013) do not mention the use of oxidized LDL triple marker test.

Berge et al (2007) examined if common prothrombotic mutations are more prevalent in patients with atrial fibrillation who have had a stroke than in healthy controls. These researchers also wanted to assess whether early recurrent
ischemic cerebrovascular events were more frequent among carriers of the factor V Leiden or the prothrombin gene mutations than among others. These investigators used a case-control design with 367 patients with acute ischemic stroke and atrial fibrillation (cases) and 482 healthy blood donors (controls). All mutations were detected with conventional polymerase-chain reaction protocols. The odds ratios for carriers of the factor V Leiden, prothrombin gene 20210GA, methylenetetrahydrofolate reductase 677CT, or platelet glycoprotein IIIa 1565TC (Pl(A2)) mutation were 0.91, (95 % CI: 0.51 to 1.59), 2.25 (95 % CI: 0.61 to 8.90), 0.83 (0.61 to 1.13), and 0.79 (0.57 to 1.10), respectively. Early recurrent ischemic stroke and total recurrent ischemic cerebrovascular events were slightly more frequent among carriers of the factor V Leiden mutation than among non-carriers: odds ratio 1.45 (95 % CI: 0.41 to 5.1), and 1.59 (0.61 to 4.1), respectively. None of the patients with recurrent ischemic cerebrovascular events had the prothrombin gene mutation. The authors concluded that these mutations are not important risk factors for thromboembolic stroke associated with atrial fibrillation. Carriers of the factor V Leiden mutation had a small, non-significantly higher risk of early recurrent ischemic cerebrovascular events.

Guidelines from the American Stroke Association and the American Heart Association (Goldstein, et al., 2011) state that "[t]he usefulness of genetic screening to detect inherited hypercoagulable states for prevention of first stroke is not well established" and "[t]he usefulness of specific treatments for primary stroke prevention in asymptomatic patients with hereditary or acquired thrombophilia is not well established."

Also, an UpToDate review on "Overview of the risk equivalents and established risk factors for cardiovascular disease" (Wilson, 2013) does not mention the use of prothrombin mutation testing.

Bonaca et al (2012) examined if pregnancy-associated plasma protein-A (PAPP-A) is useful for risk assessment in non-ST-
segment elevation acute coronary syndrome (NSTE-ACS). These investigators measured PAPP-A at baseline in 3,782 patients with non NSTE-ACS randomized to ranolazine or placebo in the MERLIN-TIMI 36 (Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST Elevation Acute Coronary Syndromes) trial and followed for an average of 1 year. A cut-point of 6.0 μIU/ml was chosen from pilot work in this cohort. Pregnancy-associated plasma protein-A greater than 6.0 μIU/ml at presentation was associated with higher rates of cardiovascular death or MI at 30 days (7.4 % versus 3.7 %, HR: 2.01; 95 % CI: 1.43 to 2.82; p < 0.001) and at 1 year (14.9 % versus 9.7 %, HR: 1.63; 95 % CI: 1.29 to 2.05; p < 0.001). Pregnancy-associated plasma protein-A was also associated with higher rates of cardiovascular death (HR: 1.94; 95 % CI: 1.07 to 3.52, p = 0.027) and MI (HR: 1.82; 95 % CI: 1.22 to 2.71, p = 0.003) individually at 30 days. There was no difference in the risk associated with PAPP-A stratified by baseline cardiac troponin I [Accu-Tnl greater than 0.04 μg/l], p interaction = 0.87). After adjustment for cardiac troponin I, ST-segment deviation, age, sex, diabetes, smoking, hypertension, and CAD, PAPP-A was independently associated with cardiovascular death/MI at 30 days (adjusted HR: 1.62, 95 % CI: 1.15 to 2.29; p = 0.006) and 1 year (adjusted HR: 1.35, 95 % CI: 1.07 to 1.71; p = 0.012). Pregnancy-associated plasma protein-A also improved the net re-classification for cardiovascular death/MI (p = 0.003). There was no significant interaction with ranolazine. The authors concluded that PAPP-A was independently associated with recurrent cardiovascular events in patients with NSTE-ACS. They stated that this finding supported PAPP-A as a candidate prognostic marker in patients with ACS and supported continued investigation of its potential therapeutic implications.

The Digital Pulse Analyzer (DPA) provides information on arterial wall stiffness and determines the biological age of arteries in less than 3 minutes. This FDA-approved, user-friendly, non-invasive device uses a finger probe to observe the changes in pressure, blood flow, velocity and profile throughout the whole pulse wave.
According to the AVIIR Corp. (Irvine, CA), the MIRISK VP is a novel, protein-based assay that measures 7 specific, highly predictive biomarkers, which are associated with the formation of vulnerable plaque. Vulnerable plaque is responsible for an estimated 75% of all heart attacks, so detecting vulnerable plaque is key to determining a patient’s cardiac risk. The test relies on a proprietary algorithm applied to 4 clinical risk factors and 7 protein biomarker measurements, to determine who is most at risk for a heart attack or unstable angina within a 5-year period. The MIRISK VP test measures serum levels of CTACK, Eotaxin, Fas Ligand, HGF, IL-16, MCP-3, and sFas. These proteins are associated with the biology of vulnerable plaque development. Vulnerable plaque rupture in a coronary artery can cause a heart attack. The MIRISK VP has been shown to identify up to 17% of low- and 25.7% of high-risk patients in a multi-ethnic study who were initially identified in the intermediate risk group for Coronary Heart Disease (CHD) by the Framingham Risk Assessment. The test offers a significant advancement in CHD risk assessment methods. MIRISK VP can further stratify individuals in the intermediate-risk group who may actually be high- or low-risk, enabling high-risk individuals to implement therapeutic lifestyle changes and enabling low-risk individuals to avoid additional testing.

Beggs et al (2013) stated that “Aviir, Inc. is a venture-funded biotechnology company developing and commercializing laboratory tests to provide personalized information to physicians and patients, with the goal of preventing cardiovascular disease and metabolic syndromes. Leveraging advanced research, Aviir developed and launched MIRISK VP™, a risk assessment test to better identify individuals at risk of a heart attack. Aviir also offers an extensive menu of other cardiovascular and metabolic tests through its Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. Efforts are likewise focused on expanding genomics testing capability to address sudden cardiac death attributed to inherited cardiovascular diseases. This completes their integrated precision diagnostics approach that combines biomarker immunoassays with genomic and transcription
analysis, along with core clinical chemistry to deliver a comprehensive personal health solution”.

Galectin-3 is a protein that is associated with the development and progression of heart failure, including progressive fibrosis (stiffening) of the heart muscle. Testing purportedly assists in assessing the prognosis of chronic heart failure.

Cystatin C is a small protein produced by cells of the body; serum testing is proposed to diagnose impaired kidney function.

Serum sterol testing (eg, Boston Heart Cholesterol Balance Test) measures the levels of plant sterols, such as beta-sitosterol and campesterol, which supposedly indicate cholesterol absorption and potential cardiac disease risk.

Skin cholesterol test (eg, PREVU) is an in-vitro diagnostic test and the only noninvasive method to assess skin cholesterol. Suggested to assess risk of CAD in individuals with a history of heart attack or with clinical symptoms or signs of CAD.

Thromboxane metabolite(s) testing is a urine test to measure the level of thromboxane production and which is purportedly useful in identifying individuals who remain at risk of a cardiovascular event despite being on aspirin therapy.

Troponin I testing (PATHFAST cTnI-II) determines the quantity of cardiac troponin I, a protein that is integral to cardiac muscle contraction, which is purportedly elevated in the bloodstream after damage to the myocardium (heart muscle).

Singulex SMC testing for risk of cardiac dysfunction and vascular inflammation (eg, SMC Endothelin, SMC IL-6, SMC IL 17A, SMC c TnI and SMC TNF-α) – These tests are purported to measure levels of cardiac disease biomarkers that may have been previously undetectable.

Atherosclerotic cardiovascular disease (ASCVD) risk testing
(individual or panel) – These laboratory tests or panels include, but may not be limited to, c-peptide, islet cell antibodies, nonesterified fatty acids (free fatty acids), proinsulin and total insulin.

Corus CAD:

The majority of first-time coronary angiography patients do not have obstructive CAD. The Corus CAD (CardioDx, Palo Alto, CA) is a peripheral blood gene expression score (GES), consisting of 23 genes, age, and sex, which can assess obstructive CAD (at least 1 vessel with ≥ 50 % angiographic coronary artery stenosis) likelihood in non-diabetic patients.

Rosenberg et al (2010) conducted a multicenter, conducted at 30 U.S. centers, to validate the Corus CAD for diagnosis of obstructive CAD in nondiabetic patients. Blood samples were obtained prior to angiography in an independent validation cohort of 526 nondiabetic patients with a clinical indication for coronary angiography. Patients with chronic inflammatory disorders, elevated levels of leukocytes or cardiac protein markers, or diabetes were excluded from the study. Obstructive CAD was defined as 50% or greater stenosis in 1 or more major coronary arteries by quantitative coronary angiography. The area under the ROC curve (AUC) was 0.70 ± 0.02 (P < 0.001); the test added to clinical variables (Diamond-Forrester method) (AUC, 0.72 with the test vs. 0.66 without; P = 0.003) and added somewhat to an expanded clinical model (AUC, 0.745 with the test vs. 0.732 without; P = 0.089). The test improved net reclassification over both the Diamond-Forrester method and the expanded clinical model (P < 0.001). At a score threshold that corresponded to a 20% likelihood of obstructive CAD (14.75), the sensitivity and specificity were 85% and 43% (yielding a negative predictive value of 83% and a positive predictive value of 46%), with 33% of patient scores below this threshold. The authors concluded that the Corus CAD may be useful for assessing obstructive CAD in nondiabetic patients without known CAD.
In a prospective, multicenter study Thomas et al (2012) obtained peripheral blood samples for the Corus CAD before myocardial perfusion imaging (MPI) in 537 consecutive patients. Patients with abnormal MPI usually underwent invasive coronary angiography; all others had research coronary computed tomographic angiography, with core laboratories defining coronary anatomy. A total of 431 patients completed GES, coronary imaging (invasive coronary angiography or computed tomographic angiography), and MPI. Mean age was 56 ± 10 years (48% women). The prespecified primary end point was Corus CAD gene expression score (GES) receiver-operating characteristics analysis to discriminate ≥ 50% stenosis (15% prevalence by core laboratory analysis). Area under the receiver-operating characteristics curve for the Corus CAD GES was 0.79 (95% confidence interval, 0.73-0.84; P<0.001), with sensitivity, specificity, and negative predictive value of 89%, 52%, and 96%, respectively, at a prespecified threshold of ≤ 15 with 46% of patients below this score. The Corus CAD GES outperformed clinical factors by receiver-operating characteristics and reclassification analysis and showed significant correlation with maximum percent stenosis. Six-month follow-up on 97% of patients showed that 27 of 28 patients with adverse cardiovascular events or revascularization had GES >15. Site and core-laboratory MPI had areas under the curve of 0.59 and 0.63, respectively, significantly less than GES. The investigators concluded that the Corus CAD GES has high sensitivity and negative predictive value for obstructive coronary artery disease. In this population clinically referred for MPI, the Corus CAD outperformed clinical factors and MPI.

McPherson et al (2013) evaluated the clinical utility of the Corus CAD in a cardiology practice. In this study, 171 patients presenting with sable chest pain and related symptoms without a history of CAD were referred to six cardiologists for evaluation. In the prospective cohort of 88 patients, the cardiologist's diagnostic strategy was evaluated before and after gene expression score (GES) testing. The objective of the study was to measure the effect of the Corus CAD on diagnostic testing using a pre/post study design. There were 83
prospective patients evaluable for study analysis, which included 57 (69%) women, mean age 53 ± 11 years, and mean Corus CAD gene expression score (GES) 12.5 ± 9. Presenting symptoms were classified as typical angina, atypical angina, and noncardiac chest pain in 33%, 60%, and 7% of patients (n = 27, 50, and 6), respectively. After the Corus CAD, changes in diagnostic testing occurred in 58% of patients (n = 48, P < 0.001). The investigators noted that 91% (29/32) of patients with decreased testing had low GES (≤ 15), whereas 100% (16/16) of patients with increased testing had elevated GES (P < 0.001). An historical cohort of 83 patients, matched to the prospective cohort by clinical factors, had higher diagnostic test use compared with the post-GES prospective cohort (P < 0.001). The investigators concluded that the GES showed clinical utility in the evaluation of patients with suspected obstructive CAD presenting to the cardiologist's office.

Herman et al (2013) found that the use of the Corus CAD gene expression score (GES) lead to a change in diagnostic evaluation. The investigators reported on the results of the Primary Care Providers Use of a Gene Expression Test in Coronary Artery Disease Diagnosis (IMPACT-PCP) trial, a prospective study of stable, nonacute, nondiabetic patients presenting with chest pain and related symptoms at 4 primary care practices. All patients underwent GES testing, with clinicians documenting their planned diagnostic strategy both before and after GES. Of the 251 study patients, 140 were women (56%); the participants had a mean age of 56 years (standard deviation, 13.0) and a mean body mass index of 30 mg/kg(2) (standard deviation, 6.7). The mean GES was 16 (range, 1-38), and 127 patients (51%) had a low GES (less than or equal to 15). The investigators noted a change in the diagnostic testing pattern before and after GES testing in 145 of 251 patients (58% observed vs. 10% predefined expected change; P < .001). The investigators concluded that incorporation of the GES into the diagnostic workup showed clinical utility above and beyond conventional clinical factors by optimizing the patient's diagnostic evaluation.
Ladapo et al (2015) concluded that the results of a registry study demonstrated clinical utility of the Corus CAD by guiding decision making of primary care providers during assessment of symptomatic patients with suspected obstructive CAD. The REGISTRY I study measured the impact of the Corus CAD Gene Expression Score (GES) on subsequent cardiac referral decisions by primary care providers. Of the 342 stable, nonacute patients evaluated, the mean age was 55 years, 53% were female, and mean (SD) GES was 16 (±10) (range = 1-40). Low GES (≤15), indicating a low current likelihood of obstructive coronary artery disease (CAD), was observed in 49% of patients. The investigators reported that, after clinical covariate adjustment, each 10-point GES decrease was associated with a 14-fold decreased odds of cardiac referral (P < .0001). Low GES patients had 94% reduced odds of referral relative to elevated GES patients (P < .0001), with follow-up supporting a favorable safety profile.

Hochheiser et al (2014) published the results of a decision analysis model to assess the economic utility of the Corus CAD gene expression score (GES) for the diagnosis of obstructive CAD. Within a representative commercial health plan’s adult membership, current practice for obstructive CAD diagnosis (usual care) was compared to a strategy that incorporates the GES test (GES-directed care). The model projected the number of diagnostic tests and procedures performed, the number of patients receiving medical therapy, type I and type II errors for each strategy of obstructive CAD diagnosis, and the associated costs over a 1-year time horizon. Results demonstrate that GES-directed care to exclude the diagnosis of obstructive CAD prior to myocardial perfusion imaging may yield savings to health plans relative to usual care by reducing utilization of noninvasive and invasive cardiac imaging procedures and increasing diagnostic yield at ICA. At a 50% capture rate of eligible patients in GES-directed care, it is projected that a commercial health plan will realize savings of $0.77 per member per month; savings increase proportionally to the GES capture rate. The authors concluded that these findings illustrate the potential value of the Corus CAD for health plans.
and patients in an age of greater emphasis on personalized medicine.

**ST2 (Growth Stimulation Expressed Gene 2)**

According to the manufacturer, ST2 (for growth stimulation expressed gene 2) (Presage ST2 Assay) quantitatively measures the concentration of soluble ST2, and has been used to assess prognosis in patients with cardiovascular disease. ST2 is a member of the interleukin-1 (IL1) receptor family of cytokines. The manufacturer states that, in the heart, ST2 has a biological role in immunological processes and is involved in a cardiac signaling pathway, which, under healthy conditions, serves to protect the heart during pressure overload or stretch. The manufacturer states that ST2 is an emerging biomarker to predict adverse outcomes and death in individuals with established heart failure and is also a prognostic marker for future cardiovascular disease in the general population.

Published studies of ST2 have focused on its relationship to prognosis in three key areas: 1) risk of hypertension, heart failure, and cardiovascular mortality in the general population (AbouEzziddine, et al., 2012; Wang, et al., 2012); 2) its relationship with adverse outcomes in patients with heart failure (Ky, et al., 2011; Felker, et al., 2013); and 3) its prognostic value in acute coronary syndromes (Shrimpo, et al., 2002; Kohli, et al., 2012). However, there is a lack of evidence of clinical utility of ST2 and ST2 has not been incorporated in current clinical guidelines.

**Coenzyme Q Testing:**

Coenzyme Q10 (CoQ10) is a fat-soluble, vitamin-like substance required for normal mitochondrial function that occurs naturally in the body. CoQ10 is used to produce energy to fuel cell growth and maintenance. CoQ10 is also an antioxidant sold in the United States (US) as a dietary supplement. A deficiency of CoQ10 is associated with a number of diseases such as mitochondrial disease, heart failure and hypertension. Testing
CoQ10 levels has been proposed for determining CVD risk and statin-related myopathy.

**Interleukin 17A Gene Polymorphism:**

Geng et al (2015) performed a case-control study to examine the association between genetic variants of IL-17A rs2275913 and IL-17F rs763780 and the development of CAD in a Chinese population. A total of 306 individuals with CAD and 306 unaffected individuals were enrolled from the Zhengzhou People’s Hospital between May 2012 and May 2014. The IL-17A rs2275913 and IL-17F rs763780 genes were genotyped by PCR combined with a restriction fragment length polymorphism (PCR-RFLP). Logistic regression analysis revealed that individuals with the AA genotype of rs2275913 were associated with increased risk of CAD, compared to those with the GG genotype in a co-dominant model [adjusted OR = 1.96; 95 % CI: 1.10 to 3.53]. On the other hand, the AA genotype of rs2275913 was correlated with moderately increased risk of CAD compared to the GG + GA genotype (adjusted OR = 1.76; 95 % CI: 1.02 to 3.07) in a recessive model. However, no significant differences were observed between polymorphisms at the IL-17F rs763780 locus and CAD risk, in co-dominant, dominant, and recessive models. The authors concluded that the findings of this study suggested that the IL-17A rs2275913 polymorphism may affect the development of CAD; however, no significant association was observed between the IL-17F rs763780 polymorphism and risk of CAD.

Shuang et al (2015) carried out a case-control study to estimate the association between IL-17A rs2275913, rs3819025 and rs3748067 polymorphisms and development of CAD. A total of 415 patients with CAD and 448 health controls were recruited during the period of March 2013 and October 2014. Genotyping of IL-17A rs2275913, rs3819025 and rs3748067 were analyzed by PCR-RFLP. By logistic regression analysis, these researchers found that individuals with the AA genotype (OR, 2.18; 95 % CI: 1.35 to 3.56) and the GA+AA genotype (OR, 1.39, 95 % CI: 1.06 to 1.84) of rs2275913 were associated with
an increased risk of CAD when compared with the GG genotype. Individuals carrying the GA+AA genotype of rs2275913 were more likely to have a higher risk of CAD in those with hypertension and smoking habit, and the adjusted ORs (95 % CI) were 3.92 (2.13 to 6.82) and 2.74 (1.71 to 4.40). The authors concluded that the findings of this study suggested that individuals with the AA genotype and the GA+AA genotype of rs2275913 are associated with an increased risk of CAD, especially in those with hypertension and smoking habit. These findings need to be validated by well-designed studies.

Vargas-Alarcon et al (2015) evaluated the role of IL-17A gene polymorphisms as susceptibility markers for CAD in the Mexican population. Four IL-17A gene polymorphisms (rs8193036, rs3819024, rs2275913 and rs8193037) were genotyped by 5' exonuclease TaqMan assays in a group of 900 patients with premature CAD and 667 healthy controls (with negative calcium score by computed tomography), seeking associations with CAD and other metabolic and cardiovascular risk factors using logistic regression analyses. No single IL-17A polymorphism was associated with premature CAD, however 2 haplotypes (CAGG and TAGA) were significantly associated with increased risk of premature CAD (OR = 1.35, 95 % CI: 1.00 to 1.84, p = 0.018 and OR = 2.09, 95 % CI: 1.16 to 3.76, p = 0.003, respectively). Moreover, rs3819024 was associated with increased levels of visceral abdominal fat (p = 0.002) and rs8193036 was significantly associated with risk of central obesity (p = 0.020), hypertriglyceridemia (p = 0.027), and metabolic syndrome (p = 0.027) in the premature CAD group, under dominant models adjusted by age, gender, BMI, smoking history, alcohol consumption, and treatment. The authors concluded that the findings of this study suggested that IL-17A haplotypes are involved in the risk of developing premature CAD and some IL-17A polymorphisms are associated with cardiovascular risk factors in Mexican individuals with premature CAD.

Lipidomic and Metabolomic Markers:
Laaksonen (2016) summarize published data on lipidomic and metabolomic risk markers of CAD. Studies were identified from a literature search of PubMed. Published data showed that analysis of metabolites and lipids offers an opportunity to increase the knowledge of the biological processes related to development and progression of atherosclerotic coronary disease. It is evident that advanced analytical technologies are able to detect and identify a large number of molecules that may have important structural and functional roles over and above currently used biomarkers in the cardiovascular field. It is suggested in a number of reports that the novel biomarkers can be used to improve risk stratification and patient selection for different treatments. In addition, monitoring treatment safety and effectiveness as well as lifestyle changes should be facilitated by such novel markers. The authors concluded that until now a plethora of biomarker candidates associated with cardiovascular event risk have been identified, but very few have passed through clinical and analytical validation and found their way into clinical use. Consequently, the appetite of physicians to use these novel tests in daily clinical routine has not yet been truly tested.

MaxPulse Testing:

Max Pulse testing is a non-invasive way to measure pulse waveform and heart rate by means of photoelectric plethysmography. There is a lack of reliable evidence regarding the clinical value of this approach. Alnaeb, et al. (2007) described the potential uses of photoplethysmography for peripheral artery disease in research and clinical use. A number of studies have reported on measurements of arterial stiffness by a digital volume pulse analysis technique using photoplethysmography, looking at its correlation with known cardiovascular disease risk factors (Gunarathne, et al., 2008; Otsuka, et al., 2006). Hashimoto, et al. (2001) reported on the relationship between photoplethysmography and pulse wave velocity measurements in hypertensive patients. Current evidence based guidelines from leading national medical professional organizations and Federal public health agencies
have no recommendation for performing photoplethysmography for the screening or diagnosis of peripheral artery disease or other cardiovascular disease.

Stress Echocardiography for Screening of Asymptomatic Patients

The American Society for Echocardiography (ASE) recommends avoiding use of stress echocardiograms on asymptomatic patients who meet “low risk” scoring criteria for coronary disease (ASE, 2013). The ASE explains that stress echocardiography is mostly used in symptomatic patients to assist in the diagnosis of obstructive coronary artery disease. There is very little information on using stress echocardiography in asymptomatic individuals for the purposes of cardiovascular risk assessment, as a stand-alone test or in addition to conventional risk factors. See also Douglas, et al. (2011).

Toll-Like Receptor 4 (TLR4) Asp299Gly (rs4986790) Polymorphism:

Chen and colleagues (2015) stated that previous studies have shown conflicting results on the association between toll-like receptor 4 (TLR4) Asp299Gly (rs4986790) polymorphism and CAD. In a meta-analysis, these investigators evaluated the influence of TLR4 Asp299Gly polymorphism on CAD risk, CRP level and the number of stenotic coronary arteries, as well as to examine if G allele carriers would benefit more from statin treatment. PubMed, EMBASE, and CNKI databases were searched until May 2015. All the statistical tests were performed using R version 3.1.2. Odds ratio and 95 % CI were used to assess the association between TLR4 Asp299Gly polymorphism and CAD risk, the number of stenotic vessels, and the incidence of cardiovascular events according to statin-treated patients. Weighted mean difference (WMD) was calculated for the association between Asp299Gly and CRP level. Overall, 12 case-control studies with 10,258 cases and 5,891 controls were included, and no association of TLR4Asp299Gly polymorphism with CAD was found (G allele
versus A allele: OR = 0.97, 95 % CI: 0.81 to 1.17, p = 0.75; AA versus GG + AG: OR = 0.97, 95 % CI: 0.80 to 1.18, p = 0.76; GG versus AG + AA: OR = 1.08, 95 % CI: 0.57 to 2.02, p = 0.82; AG versus AA + GG: OR = 1.03, 95 % CI: 0.85 to 1.25, p = 0.74).

Also, no association was noted between Asp299Gly and CRP level (WMD = ‐0.10, 95 % CI: ‐0.62 to 0.41, p = 0.69).

Furthermore, no synergistic effect of statin and 299Gly was reported (Statin_AA versus Statin_AG/GG: OR = 1.12, 95 % CI: 1.41 to 3.09, p = 0.82). The authors concluded that the findings of this meta-analysis suggested no association of TLR4 Asp299Gly polymorphism with CAD and CRP level. It is further indicated that the G allele carriers may not benefit more from statin treatment. Moreover, they stated that further studies should include large sample size and high-quality literature to understand this issue in depth.

Appendix

Framingham Risk Scoring

Framingham risk scoring for men and women below is adapted from Appendix A of the Executive Summary of the ATPIII Report, available at the following web site:


Risk assessment for determining the 10-year risk for developing CHD is carried out using Framingham risk scoring (Table 1 for men and Table 2 for women). The 10-year risk for MI and coronary death is estimated from total points, and the person is categorized according to absolute 10-year risk as indicated in the tables.

Table 1: Estimated 10-Year Risk for Men (Framingham Point Scores)

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<tr>
<th>Age</th>
<th>Points</th>
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</tr>
<tr>
<td>20-39</td>
<td>0</td>
</tr>
<tr>
<td>20-39</td>
<td>0</td>
</tr>
<tr>
<td>20-39</td>
<td>0</td>
</tr>
<tr>
<td>20-39</td>
<td>0</td>
</tr>
<tr>
<td>20-39</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-39</td>
<td>8</td>
</tr>
<tr>
<td>20-39</td>
<td>5</td>
</tr>
<tr>
<td>20-39</td>
<td>3</td>
</tr>
<tr>
<td>20-39</td>
<td>1</td>
</tr>
<tr>
<td>20-39</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Smoker</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-39</td>
<td>0</td>
</tr>
<tr>
<td>20-39</td>
<td>0</td>
</tr>
<tr>
<td>20-39</td>
<td>0</td>
</tr>
<tr>
<td>20-39</td>
<td>0</td>
</tr>
<tr>
<td>20-39</td>
<td>0</td>
</tr>
</tbody>
</table>

**Total Cholesterol**

**HDL (mg/dL)**
<table>
<thead>
<tr>
<th>Systolic BP (mm Hg)</th>
<th>If Untreated</th>
<th>If Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥120</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>120-129</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>130-139</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>140-159</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>≥160</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Point Total</th>
<th>10-Year Risk %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 2: Estimated 10-Year Risk for Women (Framingham Point Scores)

<table>
<thead>
<tr>
<th>Age</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-34</td>
<td>-7</td>
</tr>
<tr>
<td>35-39</td>
<td>-3</td>
</tr>
<tr>
<td>40-44</td>
<td>0</td>
</tr>
<tr>
<td>45-49</td>
<td>3</td>
</tr>
<tr>
<td>50-54</td>
<td>6</td>
</tr>
<tr>
<td>55-59</td>
<td>8</td>
</tr>
<tr>
<td>60-64</td>
<td>10</td>
</tr>
<tr>
<td>65-69</td>
<td>12</td>
</tr>
<tr>
<td>70-74</td>
<td>14</td>
</tr>
<tr>
<td>75-79</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Cholesterol</th>
<th>Age 20-39</th>
<th>Age 40-49</th>
<th>Age 50-59</th>
<th>Age 60-69</th>
<th>Age 70-79</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 160</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>160-199</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>200-239</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>240-279</td>
<td>11</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Age 20-39</td>
<td>Age 40-49</td>
<td>Age 50-59</td>
<td>Age 60-69</td>
<td>Age 70-79</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Smoker</td>
<td>9</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HDL (mg/dL)</th>
<th>Points</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>≥60</td>
<td>-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 40</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Systolic BP (mm Hg)</th>
<th>If Untreated</th>
<th>If Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 120</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>120-129</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>130-139</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>140-159</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>≥ 160</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Point Total</th>
<th>10-Year Risk %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 9</td>
<td>&lt;1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>CPT Codes / HCPCS Codes / ICD-10 Codes</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by &quot;+&quot;:</td>
<td></td>
</tr>
<tr>
<td>High-sensitivity C-reactive protein (hs-CRP):</td>
<td></td>
</tr>
<tr>
<td>CPT codes covered if selection criteria are met:</td>
<td></td>
</tr>
<tr>
<td>81400 - 81408</td>
<td>Molecular pathology</td>
</tr>
<tr>
<td>86141</td>
<td>C-reactive protein; high sensitivity (hsCRP) [2 or more major risk factors, LDL 100-300 mg/dl, and intermediate risk of CVD by global risk assessment - see criteria]</td>
</tr>
<tr>
<td>ICD-10 codes covered if selection criteria are met:</td>
<td></td>
</tr>
<tr>
<td>E78.6</td>
<td>Lipoprotein deficiency [low HDL cholesterol less than 40 mg/dL]</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>F17.200 - F17.291</td>
<td>Nicotine dependence</td>
</tr>
<tr>
<td>I10 - I15.9</td>
<td>Hypertensive disease [BP 140 mmHg or higher, or on antihypertensive medication]</td>
</tr>
<tr>
<td>Z82.49</td>
<td>Family history of ischemic heart disease and other diseases of the circulatory system [premature CHD]</td>
</tr>
</tbody>
</table>

**Major risk factors [need at least 2]:**

**Apolipoprotein B (apo B):**

**CPT codes covered if selection criteria are met:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>82172</td>
<td>Apolipoprotein, each [covered for apoB - not apoA1 or apoE]</td>
</tr>
</tbody>
</table>

**ICD-10 codes covered if selection criteria are met:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E10.10 - E11.9</td>
<td>Diabetes mellitus [with 2 or more CVD risk factors - see criteria]</td>
</tr>
<tr>
<td>E78.0</td>
<td>Pure hypercholesterolemia [with 2 or more CVD risk factors - see criteria]</td>
</tr>
<tr>
<td>E78.2</td>
<td>Mixed hyperlipidemia [with 2 or more CVD risk factors - see criteria]</td>
</tr>
<tr>
<td>F17.200 - F17.291</td>
<td>Nicotine dependence [with 2 or more CVD risk factors - see criteria]</td>
</tr>
<tr>
<td>I10 - I15.9</td>
<td>Hypertensive disease [with 2 or more CVD risk factors - see criteria]</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>I20.0 - I25.9</td>
<td>Ischemic heart diseases [with 2 or more CVD risk factors - see criteria]</td>
</tr>
<tr>
<td>I25.10</td>
<td>Atherosclerotic heart disease of native coronary artery without angina pectoris [with 2 or more CVD risk factors - see criteria]</td>
</tr>
<tr>
<td>I50.1 - I50.9</td>
<td>Heart failure [with 2 or more CVD risk factors - see criteria]</td>
</tr>
<tr>
<td>Z82.49</td>
<td>Family history of ischemic heart disease and other diseases of the circulatory system [with 2 or more CVD risk factors - see criteria]</td>
</tr>
</tbody>
</table>

**Tests considered experimental and investigational for assessing CHD risk:**

**CPT codes not covered for indications listed in the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0111T</td>
<td>Long-chain (C20-22) omega-3 fatty acids in red blood cell (RBC) membranes</td>
</tr>
<tr>
<td>0126T</td>
<td>Common carotid intima-media thickness (IMT) study for evaluation of atherosclerotic burden or coronary heart disease risk factor assessment</td>
</tr>
<tr>
<td>0337T</td>
<td>Endothelial function assessment, using peripheral vascular response to reactive hyperemia, non-invasive (eg, brachial artery ultrasound, peripheral artery tonometry), unilateral or bilateral</td>
</tr>
<tr>
<td>0423T</td>
<td>Secretory type II phospholipase A2 (sPLA2-IIA)</td>
</tr>
<tr>
<td>81229</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities [not covered for cardiovascular disease risk]</td>
</tr>
<tr>
<td>81240</td>
<td>F2 (prothrombin, coagulation factor II) (eg, hereditary hypercoagulability) gene analysis, 20210G&gt;A variant</td>
</tr>
<tr>
<td>81241</td>
<td>F5 (coagulation Factor V) (eg, hereditary hypercoagulability) gene analysis, Leiden variant</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>81400</td>
<td>Molecular pathology procedure, Level 1 (e.g., identification of single germline variant [e.g., SNP] by techniques such as restriction enzyme digestion or melt curve analysis) [ ]</td>
</tr>
<tr>
<td>81401</td>
<td>Molecular pathology procedure, Level 2 (e.g., 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)</td>
</tr>
<tr>
<td>82163</td>
<td>Angiotensin II</td>
</tr>
<tr>
<td>82542</td>
<td>Column chromatography, includes mass spectrometry, if performed (e.g., HPLC, LC, LC/MS, LC/MS-MS, GC, GC/MS-MS, GC/MS, HPLC/MS), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen [not covered for cardiovascular disease risk]</td>
</tr>
<tr>
<td>82610</td>
<td>Cystatin C [not covered for cardiovascular disease risk]</td>
</tr>
<tr>
<td>82725</td>
<td>Fatty acids, nonesterified [not covered for cardiovascular disease risk]</td>
</tr>
<tr>
<td>82777</td>
<td>Galectin-3 [not covered for cardiovascular disease risk]</td>
</tr>
<tr>
<td>83006</td>
<td>Growth stimulation expressed gene 2 (ST2, Interleukin 1 receptor like-1)</td>
</tr>
<tr>
<td>83520</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified [adiponectin] [leptin] [interleukin-6 (IL-6)] [tumor necrosis factor alpha (TNF-a)] [Oxidized phospholipids] [interleukin 17] [toll-like receptor 4 (TLR4)]</td>
</tr>
<tr>
<td>83525</td>
<td>Insulin, total [not covered for cardiovascular disease risk]</td>
</tr>
<tr>
<td>83695</td>
<td>Lipoprotein (a)</td>
</tr>
<tr>
<td>83698</td>
<td>Lipoprotein-associated phospholipase A2 (Lp-PLA2)</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>83700</td>
<td>Lipoprotein, blood; electrophoretic separation and quantitation</td>
</tr>
<tr>
<td>83701</td>
<td>high resolution fractionation and quantitation of lipoproteins including lipoprotein subclasses when performed (eg, electrophoresis, ultracentrifugation) [VAP cholesterol test]</td>
</tr>
<tr>
<td>83704</td>
<td>quantitation of lipoprotein particle numbers and lipoprotein particle subclasses (eg, by nuclear magnetic resonance spectroscopy)</td>
</tr>
<tr>
<td>83719</td>
<td>Lipoprotein, direct measurement; VLDL cholesterol</td>
</tr>
<tr>
<td>83876</td>
<td>Myeloperoxidase (MPO)</td>
</tr>
<tr>
<td>83880</td>
<td>Natriuretic peptide</td>
</tr>
<tr>
<td>83883</td>
<td>Nephelometry, each analyte not elsewhere specified [retinol binding protein 4 (RBP4)]</td>
</tr>
<tr>
<td>83890 - 83913</td>
<td>Molecular diagnostics</td>
</tr>
<tr>
<td>84163</td>
<td>Pregnancy-associated plasma protein-A (PAPP-A)</td>
</tr>
<tr>
<td>84206</td>
<td>Proinsulin [not covered for cardiovascular disease risk]</td>
</tr>
<tr>
<td>84431</td>
<td>Thromboxane metabolite(s), including thromboxane if performed, urine [not covered for cardiovascular disease risk]</td>
</tr>
<tr>
<td>84681</td>
<td>C-peptide [not covered for cardiovascular disease risk]</td>
</tr>
<tr>
<td>85246</td>
<td>Factor VIII, VW factor antigen</td>
</tr>
<tr>
<td>85300</td>
<td>Clotting inhibitors or anticoagulants; antithrombin III, activity</td>
</tr>
<tr>
<td>85301</td>
<td>Clotting inhibitors or anticoagulants; antithrombin III, antigen assay</td>
</tr>
<tr>
<td>85302</td>
<td>Clotting inhibitors or anticoagulants; protein c, antigen</td>
</tr>
<tr>
<td>85303</td>
<td>Clotting inhibitors or anticoagulants; protein c, activity, and Activated Protein C (APC) resistance assay</td>
</tr>
</tbody>
</table>
85384  Fibrinogen; activity
85385  antigen
85415  Fibrinolytic factors and inhibitors; plasminogen activator
86341  Islet cell antibody [not covered for cardiovascular disease risk]
88271 - 88275  Molecular cytogenetics [genetic testing] [MIRISK VP test]
93050  Arterial pressure waveform analysis for assessment of central arterial pressures, includes obtaining waveform(s), digitization and application of nonlinear mathematical transformations to determine central arterial pressures and augmentation index, with interpretation and report, upper extremity artery, non-invasive
93895  Quantitative carotid intima media thickness and carotid atheroma evaluation, bilateral
93880  Duplex scan of extracranial arteries; complete bilateral study
93882  unilateral or limited study
93922  Limited bilateral noninvasive physiologic of upper or lower extremity arteries, (eg, for lower extremity: ankle/brachial indices at distal posterior tibial and anterior tibial/dorsalis pedis arteries plus bidirectional, Doppler waveform recording and analysis at 1-2 levels, or ankle/brachial indices at distal posterior tibial and anterior tibial/dorsalis pedis arteries plus volume plethysmography at 1-2 levels, or ankle/brachial indices at distal posterior tibial and anterior tibial/dorsalis pedis arteries with transcutaneous oxygen tension measurements at 1-2 levels) [Digital Pulse Analyzer (DPA)] [MaxPulse]
<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>93923</td>
<td>Complete bilateral noninvasive physiologic studies of upper or lower extremity arteries, 3 or more levels (eg, for lower extremity: ankle/brachial indices at distal posterior tibial and anterior tibial/dorsalis pedis arteries plus segmental blood pressure measurements with bidirectional Doppler waveform recording and analysis, at 3 or more levels, or ankle/brachial indices at distal posterior tibial and anterior tibial/dorsalis pedis arteries plus segmental volume plethysmography at 3 or more levels, or ankle/brachial indices at distal posterior tibial and anterior tibial/dorsalis pedis arteries plus segmental transcutaneous oxygen tension measurements at 3 or more level(s), or single level study with provocative functional maneuvers (eg, measurements with postural provocative tests, or measurements with reactive hyperemia [Digital Pulse Analyzer (DPA)])</td>
</tr>
<tr>
<td>99090</td>
<td>Analysis of clinical data stored in computers (eg, ECGs, blood pressure, hematologic data) [CardioVision MS-2000]</td>
</tr>
<tr>
<td>Modifier 7A</td>
<td>APOE, commonly called apolipoprotein E (cardiovascular disease or Alzheimer's disease)</td>
</tr>
<tr>
<td><strong>Other CPT codes related to the CPB:</strong></td>
<td></td>
</tr>
<tr>
<td>93454 - 93461, 93563</td>
<td>Coronary Angiography [coronary artery reactivity test]</td>
</tr>
<tr>
<td><strong>ICD-10 codes not covered for indications listed in the CPB:</strong></td>
<td></td>
</tr>
<tr>
<td>E75.21 - E75.6, E78.0 - E78.9</td>
<td>Disorders of lipoid metabolism</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>F17.200 - F17.201, F17.210 - F17.211, F17.220 - F17.221, F17.290 - F17.291</td>
<td>Nicotine dependence</td>
</tr>
<tr>
<td>I10 - I15.9</td>
<td>Hypertensive disease</td>
</tr>
<tr>
<td>I25.10 - I25.119, I25.700 - I25.9</td>
<td>Coronary atherosclerosis</td>
</tr>
<tr>
<td>Z13.6</td>
<td>Encounter for screening for cardiovascular disorders</td>
</tr>
<tr>
<td>Z82.49</td>
<td>Family history of ischemic heart disease and other diseases of the circulatory system</td>
</tr>
<tr>
<td>Z87.891</td>
<td>Personal history of nicotine dependence</td>
</tr>
</tbody>
</table>

**Carotid Ultrasound Screening:**

**CPT codes not covered for indications listed in the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>93880</td>
<td>Duplex scan of extracranial arteries; complete bilateral study</td>
</tr>
<tr>
<td>93882</td>
<td>unilaterial or limited study</td>
</tr>
</tbody>
</table>

**ICD-9 codes not covered for indications listed in the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z00.00 - Z00.01</td>
<td>Encounter for general adult medical examination without or with abnormal findings</td>
</tr>
<tr>
<td>Z01.810</td>
<td>Encounter for preprocedural cardiovascular examination</td>
</tr>
<tr>
<td>Z01.818</td>
<td>Encounter for other preprocedural examination</td>
</tr>
<tr>
<td>Z03.89</td>
<td>Encounter for observation for other suspected diseases and conditions ruled out</td>
</tr>
<tr>
<td>Z04.9</td>
<td>Encounter for examination and observation for unspecified reason</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Z09</td>
<td>Encounter for follow-up examination after completed treatment for conditions other than malignant neoplasm</td>
</tr>
<tr>
<td>Z13.220</td>
<td>Encounter for screening for lipoid disorders</td>
</tr>
<tr>
<td>Z13.6</td>
<td>Encounter for screening for cardiovascular disorders</td>
</tr>
<tr>
<td>Z48.812</td>
<td>Encounter for surgical aftercare following surgery on the circulatory system</td>
</tr>
<tr>
<td>Z82.49</td>
<td>Family history of ischemic heart disease and other diseases of the circulatory system</td>
</tr>
<tr>
<td>Z86.73</td>
<td>Personal history of transient ischemic attack (TIA), and cerebral infarction without residual deficits</td>
</tr>
</tbody>
</table>

**Homocysteine testing:**

**CPT codes covered if selection criteria are met:**

- 83090 Homocysteine

**ICD-10 codes covered if selection criteria are met:**

- E72.11 Homocystinuria
- I26.01 - I26.99 Pulmonary embolism
- I74.0 - I74.9 Arterial embolism and thrombosis [unexplained thrombotic disorders]
- I82.0 - I82.91 Other venous embolism and thrombosis [unexplained thrombotic disorders]

**ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):**

- N96 Recurrent pregnancy loss
- O03.0 - O03.9 Spontaneous abortion [recurrent pregnancy loss]
- O09.291 - O09.299 Supervision of pregnancy with other poor reproductive or obstetric history [recurrent pregnancy loss]
- O26.20 - O26.23 Pregnancy care for patient with recurrent pregnancy loss
Z13.6 | Encounter for screening for cardiovascular disorders
[assessing coronary heart disease risk]

*Corus™ CAD gene expression profile:*
No specific code

**ICD-10 codes covered if selection criteria are met:**

I. Typical Symptoms: Identifying appropriate patients for *Corus™ CAD:*

| I20.1 | Angina pectoris with documented spasm [prinzmetal angina; variant angina pectoris] |
| I20.8 - I20.9 | Other and unspecified forms of angina pectoris [includes angina decubitus] |
| R06.02 | Shortness of breath |
| R07.2 | Precordial pain |
| R07.89 | Other chest pain |
| R07.9 | Chest pain, unspecified |

II. Atypical Anginal Equivalent Symptoms: Require at least one CAD risk factor from III:

| M54.89 - M54.9 | Other and unspecified dorsalgia |
| R00.2 | Palpitations |
| R10.9 | Unspecified abdominal pain |
| R11.0 | Nausea |
| R11.11 | Vomiting without nausea |
| R11.2 | Nausea with vomiting, unspecified |
| R12 | Heartburn |
| R42 | Dizziness and giddiness |
| R53.81 - R53.83 | Other malaise and fatigue; lethargy; tiredness |

III. Common CAD Risk Factors: Patient must have at least one atypical symptom listed in II in addition to at least one risk factor in list III:

<p>| E66.01 | Morbid (severe) obesity due to excess calories |</p>
<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E66.09 - E66.1, E66.8 - E66.9</td>
<td>Obesity</td>
</tr>
<tr>
<td>E78.0</td>
<td>Pure hypercholesterolemia</td>
</tr>
<tr>
<td>E78.1</td>
<td>Pure hyperglyceridemia</td>
</tr>
<tr>
<td>E78.2</td>
<td>Mixed hyperlipidemia</td>
</tr>
<tr>
<td>E78.4 - E78.5</td>
<td>Other and unspecified hyperlipidemia</td>
</tr>
<tr>
<td>E88.81</td>
<td>Metabolic syndrome [dysmetabolic syndrome X]</td>
</tr>
<tr>
<td>F17.200 - F17.299</td>
<td>Nicotine dependence</td>
</tr>
<tr>
<td>I10</td>
<td>Essential (primary) hypertension</td>
</tr>
<tr>
<td>I25.10 - I25.119</td>
<td>Coronary atherosclerosis of native coronary artery</td>
</tr>
<tr>
<td>I65.21 - I65.29</td>
<td>Occlusion and stenosis of carotid artery</td>
</tr>
<tr>
<td>I67.2</td>
<td>Cerebral atherosclerosis</td>
</tr>
<tr>
<td>I70.0</td>
<td>Atherosclerosis of aorta</td>
</tr>
<tr>
<td>I70.1</td>
<td>Atherosclerosis of renal artery</td>
</tr>
<tr>
<td>I70.201 - I70.299</td>
<td>Atherosclerosis of native arteries of the extremities</td>
</tr>
<tr>
<td>Z82.41</td>
<td>Family history of sudden cardiac death</td>
</tr>
<tr>
<td>Z82.49</td>
<td>Family history of ischemic heart disease and other diseases of the circulatory system</td>
</tr>
<tr>
<td>Z87.891</td>
<td>Personal history of nicotine dependence</td>
</tr>
</tbody>
</table>

**ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A40.0 - A41.9</td>
<td>Systemic infections</td>
</tr>
<tr>
<td>E10.10 - E13.9</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>I21.01 - I22.9</td>
<td>ST elevation (STEMI) and non-ST elevation (STEMI) myocardial infarction</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
<td>------------------------------------------------------------------</td>
</tr>
<tr>
<td>I25.700 - I25.812</td>
<td>Coronary atherosclerosis</td>
</tr>
<tr>
<td>I25.2</td>
<td>Old myocardial infarction</td>
</tr>
<tr>
<td>I50.1 - I50.9</td>
<td>Heart failure</td>
</tr>
<tr>
<td>R56.10 - R65.11</td>
<td>Systemic inflammatory response syndrome (SIRS) of non-infectious origin without/with acute organ dysfunction</td>
</tr>
<tr>
<td>Z95.1</td>
<td>Presence of aortocoronary bypass graft</td>
</tr>
<tr>
<td>Z79.51 - Z79.52</td>
<td>Long term (current) use of steroids</td>
</tr>
<tr>
<td>Z79.899</td>
<td>Other long term (current) drug therapy [immunosuppressive agents, chemotherapeutic agents]</td>
</tr>
</tbody>
</table>

The above policy is based on the following references:


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