Clinical Policy Bulletin:
Cardiovascular Disease Risk Tests

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Policy

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

   A. Aetna considers high-sensitivity C-reactive protein (hs-CRP) testing medically necessary for member the following criteria: (i) member has 2 or more coronary heart disease (CHD) major risk factors*, an low-density lipoprotein (LDL) cholesterol levels between 100 to 130 mg/dL; and (ii) member has bee an intermediate-risk of cardiovascular disease by global risk assessment (i.e., 10 to 20 % risk of CH 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; first-degree relative less than 65 years of age)
   4. Hypertension (blood pressure [BP] of 140 mm Hg or higher, or on anti-hypertensive medicatio
   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 investigational for all other indications, including u test for the general population and for monitoring response to therapy, because its clinical value for t been established.

II. Apolipoprotein B (apo B):

   Aetna considers measurement of apolipoprotein B (apoB) medically necessary for use in high-risk persons hypercholesterolemia to assess whether additional intense interventions are necessary when LDL cholesterol reached (LDL cholesterol less than 70 mg/dL and non-HDL cholesterol less than 100 mg/dL in persons with vascular disease (CVD) or diabetes mellitus, or LDL-C less than 100 mg/dL and non-HDL cholesterol less th 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; 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 has not been established.

III. Aetna considers any of the following tests for assessing CHD risk experimental and investigational because 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 AI) (Boston Heart HDL Map panel)
G. Apolipoprotein E (apo E)
H. Apolipoprotein E genotyping
   I. B-type natriuretic peptides (see CPB 0618 - Brain Natriuretic Peptide Testing)
J. Chromosome 9 polymorphism 9p21
K. Circulating microRNAs (e.g., miR-1, miR-16, miR-26a, miR-27a, and miR-29a, miR-133a, and miR-1 inclusive list)
   L. Coronary artery reactivity test
M. Factor V Leiden
N. Fibrinogen
O. Genetic testing
P. Growth stimulation expressed gene 2 (ST2)
Q. HDL subspecies (LpAI, LpAl/AII and/or HDL3 and HDL2)
R. Homocysteine testing
S. Interleukin 6 (IL-6)
T. Interleukin 6 -174 g/c promoter polymorphism
U. Kinesin-like protein 6 (KLP6)
V. LDL gradient gel electrophoresis
W. LDL subspecies (small and large LDL particles)
   X. Leptin
   Y. Lipoprotein remnants: intermediate density lipoproteins (IDL) and small density lipoproteins
   Z. Lipoprotein(a) (Lp(a)) enzyme immunoassay
AA. Lipoprotein-associated phospholipase A2 (Lp-PLA2) (PLAC)
AB. Long chain omega-3 fatty acids composition in red blood cell
AC. Mid-regional pro-atrial natriuretic peptide
AD. MIRISK VP test
AE. Myeloperoxidase (MPO)
AF. NMR Lipoprotfle
AG. Osteoprotegerin
AH. Oxidized phospholipids
AI. Peroxisome proliferator-activated receptor
AJ. Plasminogen activator inhibitor (PAI–1)
AK. Pregnancy-associated plasma protein-A (PAPP-A)
AL. Protein C
AM. Prothrombin gene mutation testing
AN. Resistin
AO. Retinol binding protein 4 (RBP4)
AP. Tissue plasminogen activator (tPA)
AQ. Tumor necrosis factor alpha (TNF-a)
AR. Total cholesterol content in red blood cell membranes
AS. VAP cholesterol test
AT. Visfatin
AU. von Willebrand factor antigen level.

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

IV. Aetna considers homocysteine testing experimental and investigational for assessing CHD or stroke risk. Homocysteine testing may be medically necessary for the following indications: (i) evaluating persons with homocystinuria synthase deficiency; (ii) evaluating persons with coagulation disorders (e.g., unexplained thrombotic disorder, venous thrombosis or pulmonary embolism); (iii) for evaluating women with recurrent pregnancy loss (see CPB Recurrent Pregnancy Loss); and (iv) for evaluating persons with borderline vitamin B12 deficiency (see CPB 12 Therapy). 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 - Hom Testing).

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

VI. Aetna considers noninvasive measurements of arterial elasticity by means of blood pressure waveforms (e.g., MS-2000, CVProfilor, Digital Pulse Analyzer (DPA), and HDI PulseWave) and noninvasive calculation and evaluation of arterial pressure waveforms (SphygmoCor) experimental and investigational for assessing CHD risk because its 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 angina 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 syndrome, current New York Heart Association (NYHA) class III or IV congestive heart failure symptoms, a coronary revascularization, persons with suspected unstable angina, persons with systemic infections, persons with inflammatory conditions, and persons currently taking steroids, immunosuppressive agents, or chemotherapies. Corus CAD is considered experimental and investigational for all other indications.

See also CPB 0228 - Cardiac CT, Coronary CT Angiography and Calcium Scoring; CPB 0525 - Screening for Lipid

Background

Non-traditional risk factors for coronary heart disease (CHD) are used increasingly to determine patient risk, in part assumption that many patients with CHD lack traditional risk factors (e.g., cigarette smoking, diabetes, hyperlipidemia, 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...
The authors conclude that current evidence does not support the notion that non-traditional risk assessment value to traditional risk assessment. The authors explained that “for each putative risk factor, there must be prospective trials demonstrating that (i) targeting individuals with elevated levels of these risk factors for proven risk-reducing in advantages over current methods of targeting therapy (e.g., by cholesterol, diabetes, and blood pressure screening) and specifically reducing the risk factor reduces hard cardiovascular end points, such as mortality, nonfatal myocardial infarction.”

Large prospective studies support screening for traditional risk factors. In one study, Greenland et al (2003) assess antecedent risk factors among patients who suffered fatal CHD or non-fatal myocardial infarction (MI) while enrolle cohort studies involving nearly 400,000 patients (age range of 18 to 59). Follow-up ranged from 21 to 30 years. Among patients age 40 to 59 at baseline who died of CHD during the 3 studies, 90 % to 94 % of women and 87 % to 93 % 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 89 % 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 1 randomized controlled trials (RCTs) to determine the prevalence of baseline conventional risk factors among CHD 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 traditional 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 evaluation of the potential clinical utility of putative risk factors for cardiovascular disease. The assessment explains that the strongest evidence of the value of such a test is direct evidence that its measurement improves patient outcomes. In the absence of such evidence, the assessment of the potential clinical utility of a test requires understanding a chain of logic and the evidence supporting those links in the chain. The potential for clinical utility assessing cardiovascular disease risk lies in following a chain of logic that relies on evidence regarding the ability to predict cardiovascular disease beyond that of current risk prediction methods or models, and evidence regarding prediction to treatment of cardiovascular disease. In order to assess the utility of a test in risk prediction, specific requirements regarding patient management based on the test results should be stated. The assessment notes that another factor 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 not currently part of the Framingham risk model. Risk factors not currently part of the Framingham model (i.e., non-traditional factors include high sensitivity CRP (hs-CRP), ankle-brachial index (ABI), leukocyte count, fasting blood glucose level, percutaneous coronary angiography, 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 published literature and found the availability and validity of the evidence varied considerably (USPSTF, 2009). The 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 data are not available to determine whether they benefit from additional treatments. In addition, there is not enough available about the benefits and harms of using non-traditional risk factors in screening. Potential harms include lifethreatening 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 therapy (USPSTF, 2009).

High sensitivity C-reactive protein (hs-CRP):
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 as an independent risk factor for coronary artery disease (CAD). Of current inflammatory markers identified for use in practice. A Writing Group convened by the American 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, therapy (e.g., drug therapies with lipid-lowering, anti-platelet, or cardio-protective agents), or therapy (e.g., drug therapies with lipid-lowering, anti-platelet, or cardio-protective agents). Group noted, however, that the benefits of such therapy based on this strategy remain uncertain. High-sensitivity hs-CRP is necessary in high-risk patients who have a 10-year risk of greater than 20%, as these patients already qualify for interventions. Individuals at low-risk (less than 10% per 10 years) will be unlikely to have a high-risk (greater than 10% through hs-CRP testing. The Writing group recommended screening average risk (10-year risk less than 10%) for purposes of cardiovascular risk assessment. The Writing Group stated that hs-CRP also may be useful in estimating patients who need secondary preventive care, such as those with stable coronary disease or acute coronary syndrome who have undergone percutaneous coronary interventions. The Writing Group posited that this information may be 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 recommend, without measuring hs-CRP, the aggressive application of secondary preventive interventions. The Writing Group recommends that measurement of hs-CRP be performed twice (averaging results), optimally 2 weeks apart, fasting or metabolically stable patients. Patients with an average hs-CRP level greater than 3.0 mg/dL are considered to be at high relative risk, and patients with an hs-CRP level of 1.0 and 3.0 mg/L are at average relative risk. If the hs-CRP level is greater than 10 mg/dL, the Writing Group recommends measurement of inflammatory markers other than hs-CRP (cytokines, other acute-phase reactants) for determination in addition to hs-CRP.

In an analysis of Women’s Health Study participants, including hs-CRP in cardiovascular disease (CVD)-risk prediction models that include or do not include hs-CRP. The models were applied to 15,048 Women’s Health Study participants. The models were age 45 or older and free of cardiovascular disease and cancer at baseline. During a mean follow-up of 10 years, the 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 model without CRP. Adding CRP to the model substantially improved predictive accuracy for women with an initial risk of at least 5%. The gain in accuracy was greatest among women initially classified in the 5% to 9.9% risk range: women were re-classified in a more accurate risk category when CRP was included in the risk-prediction model (11% of those with CRP lower than 5% and 9.5% of those with CRP lower than 9.5% moved up a risk category (to 10% to 19.9%)). Accounting for the pre-existing risk factors, smoking, and high blood pressure lessened the predictive contribution of CRP but still left CRP ahead of any cholesterol (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 (2000) assessed the risk for CVD according to levels of 4 inflammatory markers: hs-CRP, serum amyloid A, interleukin-6, and high-sensitivity C-reactive protein (hs-CRP). Homocysteine and several lipid and lipoprotein fractions (including total cholesterol and HDL cholesterol) were measured. Outcomes include fatal MI, stroke, or coronary revascularization procedures. Overall, hs-CRP showed the strongest univariate association with cardiovascular disease. Although several other markers studies were univariate predictors of CVD, hs-CRP was the only 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 screening or global risk assessment. While statin therapy and other interventions can reduce hs-CRP, whether or not reductions can actually prevent cardiovascular events is being investigated. This is in agreement with the observation...
Ballantyne (2005) who stated that studies are now under way to evaluate if targeting patients with high CRP and low will have any impact on future cardiovascular events and survival and whether changes in CRP correlate to event

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

A meta-analysis found that hsCRP concentration has continuous associations with the risk of coronary heart diseas stroke, and vascular mortality (Emerging Risk Factors Collaboration, 2010). Investigators assessed the associatio concentration with risk of vascular and non-vascular outcomes under different circumstances. Investigators meta-records of 160,309 people without a history of vascular disease (i.e., 1.31 million person-years at risk, 27,769 fatal disease outcomes) from 54 long-term prospective studies. Within-study regression analyses were adjusted for with variation in risk factor levels. The investigators found that log(e) hs-CR concentration was linearly associated wit conventional risk factors and inflammatory markers, and nearly log-linearly with the risk of ischemic vascular disea vascular mortality. Risk ratios (RRs) for coronary heart disease per 1 standard deviation higher log(e) hs-CR concentration higher) were 1.63 (95 % confidence interval (CI): 1.51 to 1.76) when initially adjusted for age and sex only, and 1.3 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; 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 morta investigators noted that RRs were largely unchanged after exclusion of smokers or initial follow-up. After further a fibrinogen, the corresponding RRs were 1.23 (1.07 to 1.42) for coronary heart disease; 1.32 (1.18 to 1.49) for ische (1.18 to 1.52) for vascular mortality; and 1.34 (1.20 to 1.50) for non-vascular mortality. The investigators conclude concentration has continuous associations with the risk of coronary heart disease, ischemic stroke, vascular morta several cancers and lung disease that are each of broadly similar size. The investigators noted that the relevance a range of disorders is unclear. The investigators found that associations with ischemic vascular disease depend c conventional risk factors and other markers of inflammation.

According to guidelines from the National Academy of Clinical Biochemistry (2009), if global risk is intermediate an remains as to the use of preventive therapies, hs-CRP measurement might be useful for further stratification into a risk category. Guidelines from the American College of Cardiology/American Heart Association (2010) also addres patients for statin therapy, stating it can be useful in men 50 years or older and women 60 years of age or older wit 130 mg/dL; not on lipid-lowering, hormone replacement, or immunosuppressant therapy; without clinical coronary h 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 bein men older than 50 years and women older than 60 years of age who are at intermediate risk (10% to 19%) accordi Framingham risk score and who do not otherwise qualify for lipid-lowering therapy (i.e., if their LDL-C is less than 3 guidelines explain that the rationale for measuring hs-CRP specifically in these individuals is that we now have cla the benefit of statin therapy in such individuals, if their hs-CRP is greater than 2.0 mg/L. The guidelines found that d the JUPITER study show that statin therapy reduces cardiovascular events (hazard ratio 0.56 [95% CI 0.46 to 0.69 guidelines note, because hs-CRP can be elevated during acute illness, clinical judgment should be exercised in the any single measurement of hs-CRP. Canadian Cardiovascular Society guidelines (2013) state that those subjects
JUPITER criteria (men greater than 50 years and women greater than 60 years of age and CRP greater than or equal to 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 indicated that there is currently no clinical role for measuring CRP routinely in children when assessing or ruling out CVD risk factors. The AHA statement explains that, although numerous studies suggest that CRP is elevated in children, it is not yet clear whether CRP measurement is of clinical relevance in children.

An American Heart Association statement on nontraditional risk factors and biomarkers in cardiovascular disease et al., 2011) stated: "The AHA statement explains that, although numerous studies suggest that CRP is elevated in children, it is not yet clear whether CRP measurement is of clinical relevance in children."

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An National Heart Lung and Blood Institute (2012) guideline on cardiovascular disease risk in children and adolescents stated: "There is 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 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 low-risk individuals and high-risk patients to assess 10-year risk of CVD. The guideline included a weak recommendation for the use of hs-CRP to patients with a standard risk assessment that is borderline, or in those with an LDL-C concentration less than 130 mg/dL.

Lipoprotein (a) enzyme immunoassay

The lipoprotein(a) (Lp(a)) enzyme immunoassay have been promoted as an important determinant of CHD risk, an 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 Lp(a) risk factor modification would improve patient-oriented health outcomes (Pejic and Jamieson, 2007). Furthermore, Lp(a) is a strong independent risk factor for CHD and has some limitations.

Prospective studies that evaluated Lp(a) as a predictor of cardiovascular events have had conflicting results. Some suggested that Lp(a) was an independent risk factor for CHD (Bostom et al, 1994; Bostom et al, 1996; Schaefer et al, 1997; Wald et al, 1994; Cremer et al, 1994; Schwartzman et al, 1998; Ariyo et al, 2003; Shai et al, 2003), while no significant association (Coleman et al, 1992; Ridker et al, 1993; Jauhiainen et al, 1991; Cantin et al, 1998; Nish et al, 2000). The meta-analysis of 5,436 patients followed for at least 1 year concluded that elevated Lp(a) is associated with increased risk of CAD (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 has some limitations." Although they identified moderate evidence for its role as an independent risk factor, they found that Lp(a) information on its incremental risk, and no prospective clinical outcome studies evaluating its role in management.

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Although some studies have linked elevated serum levels of Lp(a) to cardiovascular risk, the clinical utility of this marker is not yet established. Suk Danik et al (2006) analyzed data available from a cohort of about 28,000 participants followed for at least 1 year. Blood samples that had been frozen at study entry were tested for lipoprotein(a), and incident events were documented during the follow-up period. A total of 26 % of the women had lipoprotein(a) levels greater than or equal to 3.5 mmol/L, which is the level currently considered to confer increased cardiovascular risk. However, only the women in the high...
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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) level had prognostic value for stroke and death in men, but not for CHD in men or for any major vascular outcome in women even the links for stroke and death in men were evident only in the highest compared with the lowest quintile, not in quintiles. Ariyo et al (2003) prospectively studied 3,972 Cardiovascular Health Study participants (minimum age of 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 in the lowest quintile (hazard ratio [HR], 1.47); thus, a threshold effect was seen. Overall, women with the highest Lp(a) levels were those who had lipoprotein(a) levels at or above the 90th percentile and LDL-C levels a median. These findings indicate that routinely measuring lipoprotein(a) is of little benefit for most women. However, testing might be helpful in the clinical management of women who are at particularly high-risk or who have already had a cardiovascular event despite having few or no traditional risk factors. Since lipoprotein(a) is not decreased by lipid therapies, the mainstay of therapy for cardiovascular risk is still aggressive control of LDL-C levels with a statin or of a woman’s lipoprotein(a) level.

A meta-analysis found independent but modest associations of Lp(a) concentration with risk of CHD and stroke (EFactors 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 vascular morbidity and/or cause-specific mortality published between January 1970 and March 2009. Individual risk estimates for each of 126,634 participants in 36 prospective studies. During 1.3 million person-years of follow-up, 2,361 fatal or non-fatal vascular disease outcomes or non-vascular deaths were recorded, including 9,336 CHD outcomes, 338 hemorrhagic strokes, 751 unclassified strokes, 1,091 other vascular deaths, 8,114 nonvascular death of unknown cause. Within-study regression analyses were adjusted for within-person variation and combined using A genetic association study identified 2 single nucleotide polymorphisms that were strongly associated with both a low Lp(a) lipoprotein and an increased risk for coronary artery disease, providing support for a causal role of Lp(a) lipoprotein (Clarke et al, 2009). Investigators assessed 2,100 candidate genes in 3,145 case patients with CAD and 3,352 controls. Two single nucleotide polymorphisms (SNPs) mapped to 3 chromosomal regions (6q26-27, 9p21, and 1p13) associated with both were significantly associated with CAD risk. An accompanying editorial (Katherisan, 2009) stated: “Although the apolipoprotein(a) lipoprotein in risk assessment remains a subject of debate, there is likely to be increased enthusiasm for plasma Lp(a) lipoprotein levels (and possibly LPA genetic variants) to assess the risk of coronary disease. Additional testing might be helpful in the clinical management of women who are at particularly high-risk or who have already had a cardiovascular event despite having few or no traditional risk factors. Since lipoprotein(a) is not decreased by lipid therapies, the mainstay of therapy for cardiovascular risk is still aggressive control of LDL-C levels with a statin or 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) level had prognostic value for stroke and death in men, but not for CHD in men or for any major vascular outcome in women even the links for stroke and death in men were evident only in the highest compared with the lowest quintile, not in quintiles. Ariyo et al (2003) prospectively studied 3,972 Cardiovascular Health Study participants (minimum age of 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 in the lowest quintile (hazard ratio [HR], 1.47); thus, a threshold effect was seen. Overall, women with the highest Lp(a) levels were those who had lipoprotein(a) levels at or above the 90th percentile and LDL-C levels a median. These findings indicate that routinely measuring lipoprotein(a) is of little benefit for most women. However, testing might be helpful in the clinical management of women who are at particularly high-risk or who have already had a cardiovascular event despite having few or no traditional risk factors. Since lipoprotein(a) is not decreased by lipid therapies, the mainstay of therapy for cardiovascular risk is still aggressive control of LDL-C levels with a statin or of a woman’s lipoprotein(a) level.

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In a nested case-control study, lipoprotein(a) was found to add little to standard lipid measures and CRP in predicting peripheral arterial disease. Ridker et al (2001) had access to baseline plasma samples from 14,916 healthy men from the Health Study. Samples from 140 cases who developed symptomatic peripheral arterial disease (PAD) during 9-year follow-up 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 total cholesterol (TC) to HDL cholesterol was the strongest lipid predictor (adjusted relative risk, 3.9; 95% CI: 1.7 to 6.9) and the strongest non-lipid predictor (adjusted RR, 2.8; 95% CI: 1.3 to 5.9). In a separate analysis of which novel biomarker 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 were additionally tested for association with LDL cholesterol, apolipoprotein A-1, and apolipoprotein B-100.

No universally accepted, standardized method for determination of 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 heterogeneity (Tate et al, 1998; Tate et al, 1999; Marcovina et al, 2000). Lipoprotein(a) is unaffected by most available lipid-lowering therapies, with the exception of high-dose nicotinic acid, which is often used to lower elevated LDL-C. This has made it difficult to demonstrate that Lp(a) plays a direct role in vascular disease, since large-scale controlled studies examining the reduction of Lp(a) and hard cardiovascular end points have not been performed. Lastly, the predictive value of Lp(a) measurement is additive to that of traditional screening methods for global risk assessment.

There is no uniform guideline recommendation for the use of Lp(a) in assessment of cardiovascular disease risk. The Preventive Services Task Force (USPSTF, 2009) does not recommend the use of Lp(a) for cardiovascular screening (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 by the National Academy of Clinical Biochemistry (Cooper et al, 2009) stated that lipoprotein (a) is warranted for primary prevention and assessment of cardiovascular risk. However, if risk is intermediate (10% to 20%) uncertainty remains as to the use of preventive therapies such as statins or aspirin, then lipoprotein (a) measurement is recommended at the physician’s discretion.” The assessment also stated that, after global risk assessment, lipoprotein (a) measurement in patients with a strong family history of premature CVD may be useful for identifying individuals having a genetic predisposition to CVD. The assessment stated, however, that benefits of therapies based on lipoprotein (a) concentrations are unconfirmed.

A consensus statement by the American College of Cardiology (ACC) and the American Diabetes Association (ADA, 2008) concluded that the clinical utility of routine measurement of Lp(a) is unclear, although more aggressive control of 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 ischemic stroke, although there is no randomized intervention showing that reducing Lp(a) decreases CVD risk. The assessment also stated that there is no justification for screening the general population for Lp(a) at present, and no evidence of a benefit of treatment. Lipoprotein (a) levels for evaluating the effects of treatment. The assessment also stated that population routine measurement of lipoprotein (a) is not warranted.

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Canadian Cardiovascular Society guidelines (2013) state that measurement of Lp(a) might be of value in addition to particularly in individuals with a family history of premature vascular disease and familial hypercholesterolemia. The however, make no recommendation for use of Lp(a) in cardiovascular disease risk assessment.
Guidelines from the American Academy of Clinical Endocrinology (2012) state that testing for lipoprotein (a) is not recommended, although it may provide useful information to ascribe risk in white patients with CAD or in those with a family history of early CAD.

Guidelines from the National Heart Lung and Blood Institute (2012) on cardiovascular disease in children and adolescents state that there is currently no medication therapy specific for elevated Lp(a), and similar to isolated low HDL-C levels, focus on addressing other risk factors and on more aggressively managing concomitant elevations of LDL-C, TG, and adults, niacin will lower Lp(a) approximately 15 percent, but this has not been studied in children.

O'Donoghue et al (2014) evaluated the prognostic utility of Lp(a) in individuals with CAD. Plasma Lp(a) was measured in 3 studies; data were then combined with 8 previously published studies for a total of 18,971 subjects. Increasing levels of Lp(a) were not associated with the risk of CV events when modeled as a continuous variable (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 combined with previously published studies of Lp(a) in secondary prevention, subjects with Lp(a) levels in the highest quintile had an increased risk of CV events (OR: 1.40, 95% CI: 1.15 to 1.71), but with significant between-study heterogeneity (p < 0.001). The authors concluded that Lp(a) is significantly associated with the risk of CV events in patients with established disease, but the heterogeneity across studies remains uncertain. The authors stated that "although the current study demonstrates that patients with established disease have a high level of Lp(a) are at an increased risk of subsequent MACE, the marked heterogeneity between studies regarding the value of Lp(a) as a clinically useful biomarker for risk assessment, particularly among patients with wLDL cholesterol. Moreover, although Lp(a) may directly contribute to CHD, there is currently insufficient evidence to sug

Apo [Apolipoprotein] B testing:

Each LDL particle has one molecule of apo B per particle. Therefore, the apo B concentration is an indirect measure of the number of LDL particles, in contrast to LDL cholesterol, which is simply a measure of the cholesterol contained within LDL. Because apo B is a marker for LDL particle number, the greater or higher the apo B level suggests an increased number of 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 atherosclerotic disease. Accord guidelines, high-risk persons are those with known CVD, diabetes, or multiple CVD risk factors (i.e., smoking, history of premature CVD). The American Association of Clinical Chemistry has issued similar recommendations regarding 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. In this study, 12,461 patients with acute MI from the world's major regions and ethnic groups were compared with sex-matched controls to assess the contributions of various cardiovascular risk factors. Investigators obtained samples from 9,345 cases and 12,120 controls and measured cholesterol fractions and apolipoproteins to determine predictive values. Ratios were stronger predictors of MI than were individual components, and apolipoproteins were stronger predictors than their cholesterol counterparts. The apo B:apo A-1 ratio was the strongest predictor, with a population-attributable risk of 37% for LDL/HDL and 32% for total cholesterol/HDL. A 1-standard-deviation increase in apo B:apo A-1 associated with an odds ratio of 1.59 for MI, compared with 1.17 for an equivalent increase in total cholesterol/HDL 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 recent study found apo B and apo A-I, the main structural proteins of atherogenic and antiatherogenic lipoproteins and particles, adds...
measures of CAD risk assessment and discrimination in the general population. van der Steeg et al (2007) measured 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 healthy. Occurrence of CAD during follow-up was determined using a regional health authority database (hospital or Office of National Statistics records (deaths). The apo B:apo A-I ratio was associated with future CAD events in a traditional lipid values, including total cholesterol:HDL cholesterol ratio (adjusted odds ratio, 1.85), and independent Framingham risk score (OR, 1.77). However, the apo B:apo A-I ratio did no better than lipid values in discriminating individuals who would and would not develop CAD, and it added little to the predictive value of the Framingham risk 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 in general population, and that measuring total cholesterol and HDL appears to suffice to determine heart disease risk (2007). Investigators used a variety of techniques to evaluate the relative utility of apo B, apolipoprotein A-1 (apo A cholesterol, HDL cholesterol, LDL cholesterol, non-HDL cholesterol, and 3 lipid ratios in determining risk for CHD, 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, 19 in men, elevations in non-HDL cholesterol, apo B, total cholesterol:HDL ratio, LDL:HDL ratio, and apo B:apo A-1 ratio were 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 increased CHD. In sex-specific analyses, elevated LDL and total cholesterol were not significantly associated with in either men or women, perhaps owing to the lack of statistical power of these substudies. In men, total cholesterol and apo B:apo A-1 ratios both improved reclassification of 10-year risk for CHD; however, the difference between the two was not statistically significant.

Canadian Cardiovascular Society guidelines (2009, 2013) recommend apoB as the primary alternate target to LDL cholesterol explain that, based on the available evidence, many experts have concluded that apoB is a better marker than LDL cholesterol, and a better index of the adequacy of LDL-lowering therapy than LDL-C. The guidelines also not appear to be less laboratory error in the determination of apoB than LDL-C, particularly in patients with hypertriglyceridemia clinical laboratories could easily and inexpensively provide standardized measurements of apoB. The guidelines suggest that all experts are fully convinced that apoB should be measured routinely and, in any case, apoB is not presently the most clinical laboratories. Consequently, a substantial educational effort for patients and physicians would be required to effectively introduce apoB into widespread clinical practice. The guidelines conclude that, despite these reservations, that physicians who wish to use apoB in their clinical care should be encouraged to do so. Furthermore, the compromise approach represents a positive transitional phase in the assessment of lipid parameters to improve the clinical measurement of apoB. The guidelines state that apoB target for high-risk subjects is less than 80 and less than 100 mg/dL for subjects with very high or high CVD risk, respectively.

Guidelines from the British Columbia Medical Services Commission (2008) states that apolipoprotein B (apoB) should be measured for follow-up testing in high-risk patients who are undergoing treatment for hypercholesterolemia (but not for other 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 succeed of LDL-C–lowering therapy. The guidelines note that LDL particle number as reflected by apo B is a more effective introduction of apoB into widespread clinical practice. The guidelines conclude that, despite these reservations, that physicians who wish to use apoB in their clinical care should be encouraged to do so. Furthermore, the compromise approach represents a positive transitional phase in the assessment of lipid parameters to improve the clinical measurement of apoB. The guidelines state that apoB target for high-risk subjects is less than 80 and less than 100 mg/dL for subjects with very high or high CVD risk, respectively.

Guidelines from the American Association of Clinical Endocrinologists (2012) recommend apo B measurements to success of LDL-C–lowering therapy. The guidelines note that LDL particle number as reflected by apo B is a more 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 parallel with LDL cholesterol, apoB can be substituted for LDL cholesterol, but it does not add further to the risk guidelines found that, based on the available evidence, it appears that apoB is a similar risk marker to LDL cholesterol index of the adequacy of LDL-lowering therapy. Also, there appears to be less laboratory error in the determination of 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 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 measurements in the general population.

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

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

A Working Group of the American Association for Clinical Chemistry (Cole, et al., 2013) found that, in most studies, LDL particle number were comparable in association with clinical outcomes, and nearly equivalent in their ability to predict cardiovascular disease. The Working Group stated that apo B appears to be the preferable biomarker for guideline decision-making 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 children and adolescents (2011) stated that "In terms of other lipid measurements: (i) at this time, most but not all studies showed that measurement of apolipoprotein B (apoB) and apolipoprotein A-1 (apoA-1) for universal screening provides no additional benefit over measuring non-HDL-C, LDL-C, and HDL-C; (ii) measurement of lipoprotein(a) (Lp[a]) is useful in the assessment of residual risk with both hemorrhagic and ischemic stroke; (iii) in offspring of a parent with premature CVD and no other identifiable risk factors, high apoB, apoA-1, and Lp(a) have been noted; and (iv) measurement of lipoprotein subclasses and their lysosomal function 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 cardiovascular risk in an individual patient without known cardiovascular disease” (Wilson 2014b) do not mention the measurement of markers of cholesterol production (lathosterol and desmosterol), absorption (beta-sitosterol, campesterol, and cholestanol), or apo B as management tools.

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).

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Apolipoprotein E (apo E) testing:

Apolipoprotein E (apo E) is one of the major apolipoproteins of VLDL. Apo E is essential in the metabolism of chylomicrons and very-low-density lipoproteins and helps to clear chylomicrons and very-low-density lipoproteins. Apo E has been studied for many years in the context of CVD. Apo E polymorphisms have functional effects on lipoprotein metabolism, and have been studied associated with elevated cholesterol levels and lipid derangements. The common isoforms of apolipoprotein E (apoE4, apoE3, and apoE2) have been found to be determinants of plasma lipid concentrations, and 1 allele of the apoE gene, the epsilon4 allele, is being investigated as a risk factor for Alzheimer’s disease and stroke.

Several small studies and an earlier review have demonstrated variation in cholesterol levels and coronary disease with apo E isoforms. The literature on apo E and CVD was reviewed by Eichner et al (2002); the investigators concluded that the epsilon4 genotype yields poor predictive values when screening for clinically defined atherosclerosis despite positive, but...
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 for those carrying the epsilon4 allele. Humphries et al. (2001) investigated whether the effect of smoking on coronary heart disease affected by APOE genotype. The investigators enrolled 3,052 middle-aged men who were free of coronary heart disease and were included in a prospective cardiovascular surveillance in the second Northwick Park Heart Study (NPHSII). Compared with never-smokers, the 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), independent of other classic risk factors. In never-smokers, risk was closely similar in men with different genotype 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 the risk for carrying the epsilon4 allele was 0.84 (0.40 to 1.75) and 3.17 (1.82 to 5.50), respectively, with no significant difference between the epsilon2 carriers. For the epsilon3 group, the genotype effect on risk was no longer significant after adjustment for other factors (including plasma lipids). However, even after adjustment, smokers who were carriers of the epsilon4 allele significantly raised risk of coronary heart disease compared with the non-smoking group (2.79, 1.59 to 4.91, epsilon interaction p = 0.007). An accompanying editorial pointed out that it is important to determine how much of the variation in CHD is attributable to the effects of apoE, in order to evaluate the importance of screening for apoE genotype (Wa, 2001).

Bennett et al. (2007) conducted a meta-analysis to assess the relation of apo E genotypes to LDL cholesterol (LDL-C) levels. They identified 82 studies of lipid levels (involving data on some 86,000 healthy participants) and 52 studies of coronary outcomes (involving data on some 38,000 cases and 83,000 controls) from both published and unpublished sources. Pooling the lipid studies, researchers found a roughly linear relation toward increasing LDL-C levels when apo E alleles 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. In the coronary outcome studies, when the researchers used patients with the most common allele -- 3/3 -- as a referent, 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. Compared with individuals with the most common allele, those with the 2/2 genotype appeared to have a 20% lower risk of heart disease, while those with the 4/4 genotype appeared to have a slightly higher risk. A commentator stated that it is 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 -- unexpected (Wa, 2007).

Available evidence indicates that apo E genotype is a poor predictor of ischemic stroke. Sturgeon and colleagues evaluated the impact of apo E genotype alterations on ischemic stroke risk, as previous studies examining whether apo E genotype alterations 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 stratifying analyses by sex and race and adjusting for non-lipid risk factors for stroke, no significant relation between a stroke was identified, except for a lower risk associated with APOE-epsilon-2 compared with APOE-epsilon-3 in black participants. 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 its usefulness is still being researched. Guidelines from the American Association of Clinical Endocrinologists (2012) recommend that assessment of apo Al "may be useful in certain cases." The AACE guidelines state that a patient with low HDL-C suggests the existence of an adequate number of HDL-C particles that contain less cholesterol and an indication of less risk.

Homocysteine testing:

Homocysteine (Hcy) is an amino acid that is normally found in the body. Studies suggest that high blood levels of Hcy may increase a person's chance of developing heart disease, stroke, and reduced blood flow to the hands and feet. It is suggested that treatment consisting of high doses of folic acid, vitamins B6 and B12 decreases a patient's Hcy levels.
decreases their risk of CVD. However, published study results in the medical literature are conflicting; therefore the Hcy testing in reducing CVD risk and improving patient outcomes has not been demonstrated. ATP III noted the u the strength of the relation between Hcy and CHD, a lack of clinical trials showing that supplemental B vitamins will 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 coronary heart, cerebrovascular and peripheral vascular disease. However, the authors found only minimal eviden contributes incrementally to risk prediction. The authors also stated that it is unclear whether elevated Hcy is caus marker of atherosclerotic vascular disease. The authors found few, if any, controlled studies to evaluate risk-reduc these 4 factors. Hackman and Anand (2003) stated “[w]hether homocysteine is causative in the pathogenesis of a related to other confounding cardiovascular risk factors, or is a marker of existing vascular disease will have to awa of a number of large, randomized controlled trials studying the effect of homocysteine-lowering vitamins on cardiov points.”

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

Prospective clinical studies have failed to demonstrate beneficial effects of Hcy- lowering therapy on CVD. An inte randomized trial involved 5,522 patients with histories of documented vascular disease (coronary, cerebrovascular with diabetes plus another risk factor. Patients received either a combination pill (containing folic acid, vitamin B6, placebo daily (HOPE 2 Investigators, 2006). After 5 years, mean Hcy levels were about 25 % lower in the vitamin placebo group. However, no significant difference was found between groups in the primary endpoint of MI, stroke death (18.8 % versus 19.8 %; p = 0.41) or in various secondary outcomes. Importantly, vitamin B supplemenation 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 pre received vitamin B supplements or placebo. During an average follow-up of 3 years, vitamin supplementation conf any clinical outcome.

More recently, a RCT found no effect of treatment with folic acid, vitamin B12 and vitamin B6 for secondary preven coronary artery disease or aortic valve stenosis (Ebbing et al, 2008). The researchers reported on a randomized, d controlled trial conducted in the 2 university hospitals in western Norway in between 1999 and 2006. A total of 3,0 participants undergoing coronary angiography were randomized. At baseline, 59.3 % had double- or triple-vessel d had stable angina pectoris, and 14.9 % had acute coronary syndromes. Study participants were randomly assigne receiving daily oral treatment with folic acid plus vitamin B12 and vitamin B6; folic acid plus vitamin B12; vitamin B6 (n = 780). The primary end point of this study was a composite of all-cause death, non-fatal acute MI, acute hospi unstable angina pectoris, and non-fatal thromboembolic stroke. Mean plasma total Hcy concentration was reduced year of treatment in the groups receiving folic acid and vitamin B12. The trial was terminated early because of con participants due to preliminary results from a contemporaneous Norwegian trial suggesting adverse effects from th During a median 38 months of follow-up, the primary end point was experienced by a total of 422 participants (13. participants (14.2 %) receiving folic acid/vitamin B12 versus 203 (13.1 %) not receiving such treatment (HR, 1.09; 9 1.32; p = 0.36) and 200 participants (13.0 %) receiving vitamin B6 versus 222 (14.3 %) not receiving vitamin B6 (H 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 B6 on total mortality or cardiovascular events. The researchers concluded that “[o]ur findings do not suppo 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 supple cardiovascular risk (Albert et al, 2008). Within an ongoing RCT of antioxidant vitamins, 5,442 women who were U. professionals aged 42 years or older, with either a history of CVD or 3 or more coronary risk factors, were enrolled double-blind, placebo-controlled trial to receive a combination pill containing folic acid, vitamin B6, and vitamin B12
placebo, and were treated for 7.3 years from April 1998 through July 2005. The primary endpoint of the study was outcome of MI, stroke, coronary re-vascularization, or CVD mortality. Compared with placebo, a total of 796 women confirmed CVD event (406 in the active group and 390 in the placebo group). Patients receiving active vitamin treatment for the composite CVD primary end point (226.9/10,000 person-years versus 219.2/10,000 person-years for the 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 (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 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 years versus 49.6/10,000 person-years; RR, 1.01; 95% CI: 0.76 to 1.35; p = 0.93). In a blood sub-study, geometry level was decreased by 18.5% (95% CI: 12.5% to 24.1%; p < 0.001) in the active group (n = 150) over that of the placebo group (n = 150), for a difference of 2.27 micromol/L (95% CI: 1.54 to 2.96 micromol/L). The researchers conclude years of treatment and follow-up, a combination pill of folic acid, vitamin B6, and vitamin B12 did not reduce a con 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 ben harm, from B-vitamin supplementation in patients with diabetic nephropathy (House et al, 2010). Hyper-homocysteine is frequently observed in patients with diabetic nephropathy. B-vitamin therapy (folic acid, vitamin B6), and vitamin B12 shown to lower the plasma concentration of Hcy. In order to determine whether B-vitamin therapy can slow progressive nephropathy and prevent vascular complications, investigators conducted a multi-center, randomized, double-blind controlled trial (Diabetic Intervention with Vitamins to Improve Nephropathy [DIVINE]) at 5 university medical centers between May 2001 and July 2007 (House et al, 2010). The study involved 238 participants who had type 1 or 2 clinical diagnosis of diabetic nephropathy. Subjects were randomly assigned to receive B vitamins containing folic acid and vitamin B12, or matching placebo. The main outcome measure was a change in radionuclide glomerular filtration between baseline and 36 months. Secondary outcomes were dialysis and a composite of MI, stroke, re-vascularization to cause mortality. Plasma total Hcy was also measured. The mean (SD) follow-up during the trial was 31.9 (14.4) months, and the data was ended early by the data and safety monitoring board. At 36 months, the mean decrease in GFR was significant vitamin recipients than in non-recipients, even though plasma Hcy levels declined substantially in treated patients controls. Treated patients also incurred roughly double the risk for adverse cardiovascular events as did controls. radionuclide GFR decreased by a mean (SE) of 16.5 (1.7) mL/min/1.73 m(2) in the placebo group compared with 

A long-term RCT involving survivors of MI found that substantial long-term reductions in blood Hcy levels with folic acid B12 supplementation did not have beneficial effects on vascular outcomes (Study of the Effectiveness of Additiona Cholesterol and Homocysteine (SEARCH) Collaborative Group, 2010). In this double-blind RCT of 12,064 survivors of MI, stroke, or CVD mortality, investig ators conducted a multi-center, randomized, double-blind controlled trial (Diabetic Intervention with Vitamins to Improve Nephropathy [DIVINE]) at 5 university medical centers between May 2001 and July 2007 (House et al, 2010). The study involved 238 participants who had type 1 or 2 clinical diagnosis of diabetic nephropathy. Subjects were randomly assigned to receive B vitamins containing folic acid and vitamin B12, or matching placebo. The main outcome measure was a change in radionuclide glomerular filtration between baseline and 36 months. Secondary outcomes were dialysis and a composite of MI, stroke, re-vascularization to cause mortality. Plasma total Hcy was also measured. The mean (SD) follow-up during the trial was 31.9 (14.4) months, and the data was ended early by the data and safety monitoring board. At 36 months, the mean decrease in GFR was significant vitamin recipients than in non-recipients, even though plasma Hcy levels declined substantially in treated patients controls. Treated patients also incurred roughly double the risk for adverse cardiovascular events as did controls. radionuclide GFR decreased by a mean (SE) of 16.5 (1.7) mL/min/1.73 m(2) in the placebo group compared with 

The authors concluded that, among patients with diabetic nephropathy, vitamins compared with placebo resulted in a greater decrease in GFR and an increase in vascular events. Comm study, Schwenk (2010) stated, "given that most other trials also have shown that B-vitamin supplementation does and CV disease, such supplements should be avoided unless patient subgroups that derive benefit are identified in trials."

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http://qawww.aetna.com/cpb/medical/data/300_399/0381_draft.html 01/28/2015
(vitamins, 405 [6.7%], versus placebo, 392 [6.5%]). An accompanying commentary by Schwenk (2010) stated: "Those of the seven prior major trials, should end what seems to be an unjustified persistence by many clinicians to supplementation to prevent CV disease. Clinical efforts should focus on modification of CV risk factors, for which improved outcomes."

These results are consistent with earlier RCTs of Hcy lowering therapy for CVD. In a multi-center double-blind ran Toole et al (2004) enrolled 3,680 patients with non-disabling, non-embolic ischemic strokes and total Hcy levels ab percentile for the North American stroke population. Patients received either high- doses of Hcy-lowering vitamins 25 mg pyridoxine, and 0.4 mg cobalamin) or low doses that would not be expected to lower Hcy significantly (20 µg, respectively). During 2 years of follow-up, mean total Hcy decreased from 13.4 µmol/L to about 11 µmol/L in the control group and changed only minimally in the intervention group. However, no reductions were noted in rates of recurrent stroke, 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 including mean plasma Hcy levels of 12 µmol/L. By 3 months, Hcy levels had decreased among folic-acid recipients and changed only minimally in the control group. By a mean follow-up of 24 months, clinical vascular events (i.e., death, MI, stroke, invasive cardiovascular surgery) had occurred at similar rates in folic-acid (12.3%) and standard-care (11.2%) recipients; the simulation among patients in the highest quartile of baseline Hcy level (greater than 13.7 µmol/L). In multi-variate analyses, 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 published multi-national RCT designed to assess the effect of Hcy-lowering therapy on the risk for arterial disease investigators studied whether daily folate (2.5 mg) and vitamins B6 (50 mg) and B12 (1 mg) affected the risk for venous thrombosis or pulmonary embolism. Subjects were 5,522 adults (age 55 years and older) with arterial vascular disease, and at least 1 other CVD risk factor. During a mean follow-up of 5 years, Hcy levels decreased more in the B-vitamin group than in the placebo group. However, the incidence of venous thromboembolism did not differ between the B-vitamin 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 a trial of Hcy therapy to prevent recurrent VTE, den Heijer et al (2007) enrolled 701 patients with recent VTE (either thrombosis or pulmonary embolism), but without major predisposing risk factors such as recent surgery or immobility at baseline. 50% of the patients had hyper-homocysteinemia (mean, 15.5 µmol/L), and 50% had normal levels (mean, 9.2 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 that was not significant -vitamin and placebo groups (5.4% versus 6.4%). In hyper-homocysteinemic patients, the incidence of recurrent thromboembolism was non-significantly higher in B-vitamin recipients than in placebo recipients (6.7% vs. 6.0 normal Hcy, the incidence of recurrent VTE was non-significantly lower in B-vitamin recipients (4.1% versus 7.0%) in hyper-homocysteinemic patients, the incidence of recurrent thromboembolism was non-significantly higher in B-vitamin recipients than in placebo recipients (6.7% versus 6.0 normal Hcy, the incidence of recurrent VTE was non-significantly lower in B-vitamin recipients (4.1% versus 7.0%) noted that their study might have been under-powered to detect a small beneficial effect. However, they also observed the protective association with venous thromboembolism might in fact be mediated by some other thrombosis is correlated with Hcy.

An American Heart Association Science Advisory (Malinow et al, 1999) has concluded: "Although there is consider epidemiological evidence for a relationship between plasma homocyst(e)ine and cardiovascular disease, not all professionals have supported such a relationship .... Until results of controlled clinical trials become available, population-wide recommendations for treatment (supplemental vitamins) is still considered experimental, pending results from inte 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 evalu-

http://qawww.aetna.com/cpb/medical/data/300_399/0381_draft.html 01/28/2015
The National Academy of Clinical Biochemistry (Cooper and Pfeiffer, 2009) stated that "we conclude that the clinical Hcy measurement for risk assessment of primary prevention of CVD is currently uncertain."

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


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

Summarizing the evidence for use of homocysteine, a European consensus guideline (2012) stated that homocysteine as an independent risk factor for cardiovascular disease. The guidelines state that magnitude of homocysteine risk is modest, and consistency is often lacking, mainly due to nutritional, metabolic (e.g. renal disease), and lifestyle factors. The guidelines note that, in addition, intervention studies using B vitamins to reduce plasma homocysteine have provided inconsistent results. The guidelines conclude that, together with the cost of the test, homocysteine is 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 event.

Veeranna et al (2011) examined if adding Hcy to a model-based on traditional CVD risk factors improves risk classification in populations. 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 endpoints and CHD mortality in the NHANES III survey using adjusted Cox-proportional hazard analysis. Reclassification of CHD events was performed using a net reclassification improvement (NRI) index with a Framingham risk score (FRS) as the baseline. Homocysteine level (greater than 15 μmol/L) significantly predicted CVD (adjusted hazard ratio [aHR] 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: 2.01 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 adjustment for traditional risk factors and CRP. The level of Hcy, when added to FRS, significantly re-classified 12.9% and 18.3% of the intermediate-risk population from the MESA and NHANES cohorts, respectively. The level of Hcy 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: 0.43 to 0.71; p < 0.001) datasets. The authors concluded that from these 2 disparate population cohorts, they found that adding 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 considered as a screening tool in primary prevention, it is imperative that further trials are conducted in low- and intermediate-risk populations with previous CVD. Only then can the real value of measuring Hcy as a nontraditional CVD risk factor or risk marker be fully appreciated.

Intermediate and small density lipoproteins:

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

The Women's Angiographic Vitamin and Estrogen (WAVE) trial (Bittner et al, 2004) examined whether hormone therapy affects RLP-C and RLP-triglyceride (TG) levels in women with coronary artery disease, and whether these factors predict progression. WAVE was a randomized, placebo-controlled, clinical trial of hormone therapy (conjugated equine estrogens plus medroxyprogesterone acetate) and antioxidants in 423 post-menopausal women with angiographic coronary angiography at 2.8 years showed no benefit with hormone therapy or antioxidants, and no interaction between the treatments was observed. 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 percentile in the Framingham cohort. In multi-variate analyses, RLP-C...
were not related to waist-hip ratio, body mass index (BMI), smoking status, or use of lipid-lowering agents. Compared to placebo, hormone therapy did not significantly reduce RLP levels. Neither baseline RLP levels nor changes in the angiographic findings at the end of the study.

The National Cholesterol Education Program Adult Treatment Panel III (ATPIII) Guidelines (2002) state that lipoproteins 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 to CHD risk are limited, and measurement with specific assays cannot be recommended for routine practice.” The conclusion, however, that the most readily available method of measuring atherogenic triglyceride-rich lipoproteins is VLDL. A consensus statement by the ACC and the ADA (Brunzell et al, 2008) noted that, although small dense LDL particles are particularly atherogenic, the association of small LDL and cardiovascular disease may simply reflect the increased risk of cardiovascular disease in patients with small LDL.

According to guidelines from the American College of Cardiology and the American Heart Association (2010), measurement of lipoprotein subclasses and their sizes by advanced lipoprotein testing has not been shown to be particularly atherogenic. A consensus statement by the ACC and the ADA (Brunzell et al, 2008) noted that, although small dense LDL is particularly atherogenic, the association of small LDL and cardiovascular disease may simply reflect the increased risk of cardiovascular disease in patients with small LDL.

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HDL subspecies:

HDL comprises several components and subfractions that also have been related to CHD risk. While HDL cholesterol is the most commonly used, HDL subfractions (lipoprotein A1 (LpA1) and lipoprotein A1/A2 (LpA1/A2)) and/or HDL3 are not recommended for routine measurement of CHD risk assessment. A consensus statement by the ACC and the ADA (Brunzell et al, 2008) noted that HDL subspecies appear to provide little clinical value beyond measurements of HDL cholesterol.

LDL subspecies (LDL particle sizes) and LDL particle number:

A number of studies have reported that both larger low-density lipoprotein (LDL) particle size and smaller LDL particles have been associated with CHD risk. It is thought that LDL subspecies at both extremes of LDL size and density distribution have a receptor affinity.

ATPIII stated that although the presence of small LDL particles has been associated with an increased risk of CHD which small LDL particles predict CHD independent of other risk factors is “controversial.” It has been argued by C (2002), based on epidemiologic evidence, that the relationship between small LDL and CHD found in some studies 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 coronary event. Large LDL particles are thought to be large because of high cholesterol ester content. However, that the relationship between LDL particle size and coronary events was not present among members of the cohort with pravastatin, perhaps because pravastatin acts by reducing the size of LDL particles. The author concluded that the patients on the basis of LDL size may not be useful clinically, since effective treatment for elevated LDL cholesterol 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 particle measured. As small dense LDL particles seem to be particularly atherogenic, assessment of particle size has intuitive importance. LDL particle concentration and LDL size are important predictors of CVD. However, the Multi-Ethnic Study of Atherosclerosis (MESA) 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 significantly related to coronary heart disease (CHD) events [Otivos et al, 2006]. The association of small LDL and reflect the increased number of LDL particles in patients with small LDL. Hence, it is unclear whether LDL particle 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 the following: "Limitations of the clinical utility of NMR measurement of LDL particle number or size include the facts that it is not widely available and that it is currently relatively expensive. In addition, there is a need for more independent validation of the method and whether its CVD predictive power is consistent across various ethnicities, ages, and clinical lipid metabolism."

An assessment by the California Technology Assessment Forum (CTAF) (Walsh, 2008) of LDL particle number as assessed by nuclear magnetic resonance concluded that this test did not meet CTAF's assessment criteria. The CTAF assessment stated that 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 adequately answer the question of how strongly LDL subfraction information is associated with CVD [cardiovascular disease] relation to other known and putative risk factors. In summary, none of the LDL subfraction measurements have demonstrated the ability to discriminate between individuals who are at higher versus lower risks of cardiovascular disease compared to commonly used predictors, such as LDL and HDL cholesterol” (Balk et al, 2008). The AHRQ report states that to be determined if cardiac disease risk assessment and treatment decisions would be improved by adding LDL subclass (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 considered to be insufficient to show added benefit over standard risk assessment for primary prevention. The assessment found that additional data that measurement of lipoprotein subclasses over time is useful to evaluate the effects of treatment. The assessment also noted that several methods are available to assess lipoprotein subclasses, and that standardization of these methods is necessary.

According to guidelines from the American College of Cardiology and the American Heart Association (2010), measurement of lipoprotein subclasses and their sizes by advanced lipoprotein testing has not been shown to be of clinical utility in children at this time. The guidelines state that the plasma levels of VLDL-C, LDL-C, and HDL-C sizes have been determined in children and adolescents by nuclear magnetic resonance spectroscopy and by vertical 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) conclude that the role that novel CVD risk biomarkers, including advanced lipid testing, should play in risk assessment and in the development 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 concluded: “Currently, in the opinion of this Working Group on Best Practices, apo B appears to be the preferred b 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 f cholesterol, apolipoprotein B (apoB), or lipoprotein particle measurements to assess residual CVD risk in statin-trea are likely to have small LDL particles, such as people with diabetes, but it is unclear whether clinical management these measurements.

LDL gradient gel electrophoresis:

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

There is inadequate evidence that LDL subclassification by electrophoresis improves outcomes of patients with ca disease. According to the guidelines of the National Cholesterol Education Program, electrophoretic methods “can recommended as procedures of choice for measuring LDL-cholesterol.”

An assessment by the National Academy of Clinical Biochemistry on LDL particle concentration and subclasses (in measurement by gradient gel electrophoresis) (Wilson et al, 2009) concluded: “Lipoprotein subclasses, especially t concentration of small, dense LDL particles, have been shown to be related to the development of initial CHD even analyses of existing studies are generally not adequate to show added benefit over standard risk assessment for p

Measurement of LDL GGE has not been established as a clinically useful test at this time. It has not been proven determining therapy for patients with CAD or dyslipemia.

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

Angiotensin gene:

Angiotensin gene polymorphisms have been associated with CVD risk and certain forms of hypertension. Certain polymorphisms have been associated with responsiveness of BP to sodium restriction and ACE inhibitors, so that a gene may have the potential to help individualize therapy by predicting patients’ responsiveness to certain anti-hyp interventions. CardiaRisk AGT from Myriad Genetics Laboratories is a laboratory test that analyzes the angiotensin value of analyzing angiotensin gene polymorphisms in altering the management and improving outcomes of patien demonstrated in prospective clinical studies.

Fibrinogen:

Fibrinogen is a circulating glycoprotein that acts at the final step in the coagulation response to vascular and tissue epidemiological data support an independent association between elevated levels of fibrinogen and cardiovascular mortality.

In a structured evidence review, Hackman and Anand (2003) found moderate evidence that fibrinogen is an indep

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In a structured evidence review, Hackman and Anand (2003) found moderate evidence that fibrinogen is an indep
A consensus statement from the ACC and the ADA (Brunzell et al, 2008) stated that the independent predictive utility of fibrinogen measurement is unclear. A guideline from the National Academy of Clinical Biochemistry (Cushman et al., 2011) stated that: "There are sufficient data that fibrinogen is an independent marker of CVD risk; however, because of an insufficient assay standardization, and uncertainty in identifying treatment strategies, measurement is not recommended.

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

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

A European consensus guideline (2012) included a strong recommendation that fibrinogen should not be measured in 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.

Lipoprotein-associated phospholipase A2 (Lp-PLA2) (PLAC):

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). Premarket notification, the U.S. Food and Drug Administration has cleared for marketing the PLAC Test (diaDexus, 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 (Per 2001). Some large prospective clinical studies have found lipoprotein-associated phospholipase A2 (Lp-PLA2) to be an independent factor for CAD (e.g., Packard et al, 2000; Blake et al, 2001; Ballantyne et al, 2004), although another large study (W Study) found that the predictivity of Lp-PLA2 was no longer statistically significant after adjustment for other risk factors.

Other studies have failed to find an association between Lp-PLA2 and various cardiac disease endpoints (e.g., Kar Allison et al, 2006; Kardys et al, 2007; Rana et al, 2009; Oldgren et al, 2007). Rana et al (2009) examined the con-activity and abdominal obesity to the variation in Lp-PLA2 and other inflammatory biomarkers and incident CHD. A case-control study nested in the European Prospective Investigation into Cancer and Nutrition-Norfolk cohort, the associations between circulating levels or activity of lipoprotein-associated phospholipase A2 (Lp-PLA2) and other markers and CHD risk over a 10-year period among healthy men and women (45 to 79 years of age). A total of 1,079 cases (devel oped fatal or non-fatal CHD) were matched to 1,859 controls on the basis of age, sex, and enrollment period. Waist circumference, physical activity, smoking, diabetes, systolic blood pressure, low-density lipoprotein and high-cholesterol levels, and further adjusted for hormone replacement therapy in women, Lp-PLA2 was not associated with CHD risk.

A meta-analysis found Lp-PLA2 to be significantly associated with CVD (Garza et al, 2007). 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. With traditional CVD risk factors and CRP, the odds ratio was 1.60 (95% CI: 1.36 to 1.89). An accompanying editorial meta-analytic confirmation of this association is notable, clinicians must not 'jump the gun.' Important questions should be addressed before Lp-PLA2 is incorporated into clinical practice, and the authors acknowledge this fully in their discussion (Ste 2007). The editorialist explained that one of these questions is whether measurement of Lp-PLA2 yields additional information 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...
The editorialist explained that, if a patient's baseline probability of CVD is 50%, plotting an odds ratio of 1.60 on a nomogram results in a posterior probability of about 59%, a relatively small increase. "Such small changes in prob translate into changes in patient management or reclassification of patients into different risk groups." The editoria the operating characteristics of the FDA-cleared test for Lp-PLA2, the PLAC test (diaDexus Inc, San Francisco, CA adequately established (Steinberg and Mayer, 2007). The editorialist argued that decisions about the utility of a no should not be based solely on measurements of association, such as odds ratios or relative risk. Instead, clinical d should be guided by the performance characteristics of the diagnostic test that measures the biomarker. The edito test characteristics can vary significantly between patient populations. The positive and negative likelihood ratios o patients at low-, intermediate-, and high-risk of various cardiovascular outcomes need to be clarified if the test is to populations. Furthermore, prospective studies need to be performed to determine whether the use of the PLAC tes of Lp-PLA2, leads to meaningful changes in patient management. "As mentioned previously, the weak association and CVD makes this unlikely." The editorialist also explained that the fact that Lp-PLA2 is associated with CVD do be relied on as a surrogate marker of morbidity or mortality in clinical trials (Steinberg and Mayer, 2007). Clinical tr therapy will surely track Lp-PLA2 levels, but they must also measure clinical outcomes. The editorialist also questi -spread statin use, which has changed and grown considerably since many of the patients in previous studies were already offsetting the small increased risk of CVD that elevated Lp-PLA2 might confer. "This question highlights a researchers of Lp-PLA2 drug therapy -- randomized controlled trials must be performed against background therap current practice." The editorialist explained that, not until this work is done will we know if lowering Lp-PLA2 with ta therapy is good for patients. The editorialist concluded that Lp-PLA2 should not be used for screening or risk strat study. Regarding Lp-PLA2 specific drug therapy, "healthy skepticism is advised." "Responsible clinicians will resis 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-other novel risk factors to traditional risk factors (age, race, sex, HDL and total cholesterol levels, systolic BP, use agents, and smoking and diabetes status) improved prediction of incident coronary heart disease among nearly 16 years or older). The authors found that, although Lp-PLA2 showed a statistically significant increase in predictive with traditional risk factors only, this increase was not clinically important. The accompanying editorialist comments only 1 in 3 people with elevated blood pressure or cholesterol levels achieves adequate control, clinician should fo and control of traditional risk factors. The authors concluded that, for now, routine screening of Lp-PLA2 levels see

An analysis of the Atherosclerosis Risk in Communities Study, which assessed the association of 19 novel risk fac heart disease in a cohort of 15,792 adults, found that measurement of Lp-PLA2 in that population added very little predicted risk of a coronary heart disease event based on assessment of traditional risk factors (Folsom et al, 2006 PLA2 was among the novel risk factors that added the most to the area under the receiver operating curve (AUC), in a very small increase in the AUC of only 0.006. The authors concluded that routine measurement of Lp-PLA2 a markers is not warranted for risk assessment. The authors stated that, on the other hand, their findings reinforce th 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 th PLA2 and C-reactive protein to predict stroke cases in a manner that is statistically independent from traditional ris authors use data from the Atherosclerosis Risk in Communities (ARIC) Study, a high-quality prospective follow-up adults with standardized risk factor measurements as well as stored blood samples that facilitated analysis of the p predictors. As expected from prior research on stroke risk, race, hypertension, diabetes, systolic and diastolic blo triglyceride and HDL-C levels were each individually associated with higher stroke risk. The investigators reported higher Lp-PLA2 and CRP levels with increased stroke risk in statistical models adjusted for the major traditional ris highest tertile, CRP level was associated with higher stroke risk by about 2-fold, although confidence intervals were PLA2 levels in the top tertile, with adjustment for traditional risk factors and CRP, stroke risk was higher by about 2. Thus, the investigators found that the Lp-PLA2 level was a moderately strong stroke risk predictor, and its associat this study was statistically independent of traditional risk factors as well as the inflammatory marker CRP. In unadj apparently healthy middle-aged people with high levels of both CRP and Lp-PLA2 (highest tertiles of both) had a s
higher than people with low levels of both. The authors speculated that Lp-PLA2 and CRP levels may be comple- 
mentary 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 C 
level will be for improving risk prediction versus traditional risk factors alone (Greenland and O’Malley, 2005). The 
explained that, simply showing statistical independence is not adequate for demonstrating clinical utility for risk pre 
ratio and p values are useful for demonstrating statistical associations, but they fail to show whether the new mar 
ning a major impact on risk prediction or risk discrimination.” The editorialists explained that one helpful way 
additive utility of a new test is through the use of receiver operating characteristic (ROC) curves and AUC informati 
noted that, unfortunately, Ballantyne et al did not report AUC or ROC information. However, based on statistical a 
reported elsewhere, individual tests with relative risks of only 2.0 to 3.0 "are simply not capable of increasing the A 
significant degree.” The editorial concluded that "[t]o date, this search for new cardiovascular risk markers has no 
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 conventiona 
predicting future cardiovascular events in a low-to-moderate risk community-based population. Melander et al (200 
cohort study of 5,067 persons without cardiovascular disease from Malmö, Sweden, who attended a baseline exa 
1991 and 1994. Participants underwent measurement of Lp-PLA2, CRP, cystatin C, midregional proadrenomedull 
mid-regional proatrial natriuretic peptide, and N-terminal pro-B-type natriuretic peptide (N-BNP) and underwent follo 
using the Swedish national hospital discharge and cause-of-death registers and the Stroke in Malmö register for fir 
events (MI, stroke, coronary death). During median follow-up of 12.8 years, there were 418 cardiovascular and 23 
Lp-PLA2 did not have a statistically significant relationship to cardiovascular events or coronary events, and was no 
backwarded elimination models for cardiovascular events and coronary events. Models with conventional risk facto 
of 0.758 (95 % CI: 0.734 to 0.781) and 0.760 (0.730 to 0.789) for cardiovascular and coronary events, respectively 
retained in backward-elimination models were CRP and N-BNP for cardiovascular events and MR-proADM and N-
events, which increased the C statistic by 0.007 (p = 0.04) and 0.009 (p = 0.08), respectively. The investigators re 
the proportion of participants reclassified was modest (8 % for cardiovascular risk, 5 % for coronary risk). Net re-cl 
improvement was non-significant for cardiovascular events (0.0 %; 95 % CI: -4.3 % to 4.3 %) and coronary events 
0.76 % to 10.1 %). Greater improvements were observed in analyses restricted to intermediate-risk individuals (ca 
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). 
re-classification was almost entirely confined to down-classification of individuals without events rather than up-cla 
with events. In this cohort of some 5,000 participants initially free of CVD and followed almost 13 years, the novel 
proved prediction scores “only minimally,” resulting in the re-assignment of only 1 % of participants to a higher ri 
et al, 2009).

A meta-analysis found associations of circulating Lp-PLA2 mass and activity with risk of coronary heart disease, st 
under different circumstances (Lp-PLA(2) Studies Collaboration, 2010). The investigators conducted a meta-analy 
calculate risk ratios (RRs) per 1 standard deviation (SD) higher value of Lp-PLA2. The investigators found relative 
heart disease, adjusted for conventional risk factors, of 1.10 (95 % CI : 1.05 to 1.16) with Lp-PLA2 activity and 1.1 
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 1.2 
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) 
1.18) for non-vascular mortality, respectively. Although the researchers acknowledge that further research is requi 
suggest, “Randomised trials of potent reversible pharmacological inhibitors of Lp-PLA2 activity should help to 
modification of Lp-PLA2 can reverse vascular risk.” An accompanying editorial stated that these analyses suggest 
PLA2 activity is associated with higher risk of coronary heart disease (Rosenson, 2010). The editorialist noted, ho 
predictive value of Lp-PLA2 activity was weaker with higher apolipoprotein B concentrations; lower concentrations 
(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-S 
Lp-PLA2 activity) than were apolipoprotein B concentrations in the higher two tertiles (1.09 [1.01 to 1.19] and 1.11 [1-
respectively). The editorialist stated that future studies that evaluate the cardiovascular risks associated with Lp-P 
mass should at least adjust for apolipoprotein B concentrations, and small LDL-particle concentration. The edito 
theses analyses are important to fully understand the contribution of increased Lp-PLA2 activity and/or mass to futu
cardiovascular events beyond the risk obtained from quantification of LDL particles. "Clinically, the independent co
PLA2 concentrations or activity for risk stratification beyond the association with small LDL-particle concentration a
randomised trials that are designed to investigate whether selective and reversible inhibition of this pathway reduce
events."

Lp-PLA2 is also being investigated for predicting outcome in acute ischemic stroke. Elkind et al (2006) reported o
based study of stroke risk factors in 467 patients with first ischemic stroke. The study was undertaken to determine
hs-CRP and Lp-PLA2 predict risk of stroke recurrence, other vascular events, and death. The investigators found
PLA2 and hs-CRP were weakly correlated (r = 0.09; p = 0.045). High-sensitivity CRP, but not Lp-PLA2, was asso
severity. After adjusting for age, sex, race and ethnicity, history of coronary artery disease, diabetes mellitus, hype
hyperlipidemia, atrial fibrillation, smoking, and hs-CRP level, compared with the lowest quartile of Lp-PLA2, those i
quartile had an increased risk of recurrent stroke (adjusted HR, 2.08; 95 % CI: 1.04 to 4.18) and of the combined o
recurrent stroke, MI, or vascular death (adjusted HR, 1.86; 95 % CI: 1.01 to 3.42). The researchers reported that, a
counfiders, hs-CRP was not associated with risk of recurrent stroke or recurrent stroke, MI, or vascular death bu
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 an
ischemic stroke. The investigators searched Medline and EMBASE from 1966 to January 2007 for studies of blo
patients with ischemic stroke and an assessment of outcome (death, disability, or handicap). The investigators fou
blood markers that met inclusion criteria, including 1 study of Lp-PLA2 (citing Elkind et al, 2006). The researchers
although blood biomarkers might provide useful information to improve the prediction of outcome after acute ische
review showed that many studies were subject to bias. The researchers found that although some markers had so
ability, none of the studies was able to demonstrate that the biomarker added predictive power to a validated clinic
researchers concluded that the clinical usefulness of blood biomarkers for predicting prognosis in the setting of isch
yet to be established.

Few studies have investigated the role of elevated Lp-PLA2 with stroke risk (Wassertheil-Smoller et al, 2008). Wa
and colleagues (2008) assessed the relationship between Lp-PLA2 and the risk of incident ischemic stroke in 929 s
935 control subjects in the Hormones and Biomarkers Predicting Stroke Study, a nested case-control study from th
Initiative Observational Study. Mean (SD) levels of Lp-PLA2 were significantly higher among case subjects (309.0
subjects (296.3 [87.3]; p < 0.01). Odds ratio for ischemic stroke for the highest quartile of Lp-PLA2, compared with
for multiple covariates, was 1.08 (95 % CI: 0.75 to 1.55). However, among 1,137 nonusers of hormone therapy at
corresponding odds ratio was 1.55 (95 % CI: 1.05 to 2.28), whereas there was no significant association among 73
(odds ratio: 0.70; 95 % CI: 0.42 to 1.17; p for interaction = 0.055). Moreover, among non-hormone users, women w
high Lp-PLA2 had more than twice the risk of stroke (odds ratio: 2.26; 95 % CI: 1.55 to 3.35) compared with wom
biomarkers. Furthermore, different stroke cases were identified as high-risk by Lp-PLA2 rather than by CRP. The
concluded that Lp-PLA(2) was associated with incident ischemic stroke independently of CRP and traditional cardi
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
and mass, respectively, on levels and incidence of first CHD and ischemic stroke. Lp-PLA2 activity and mass were
5,393 (60 % women) subjects who participat ed in the Malmo Diet and Cancer Study cardiovascular program durin
during all, 347 subjects had an event (195 CHD and 152 ischemic strokes) during the follow-up period (mean 10.6 +/-
1.7 sex- and CV risk factors-adjusted Cox regression analysis, comparing top to bottom tertile of Lp-PLA2 activity, the
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, respect
 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 con
 elevated levels of Lp-PLA2 activity and mass, respectively, were in this study, independently of established risk fac
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
Communities (ARIC) study, to determine whether Lp-PLA2 and hs-CRP levels improved the AUC for 5-year ischem
investigators also examined how Lp-PLA2 and hs-CRP levels altered classification of individuals into low-, interme
categories compared with traditional risk factors. In a model using traditional risk factors alone, the AUC was 0.732
the biomarkers increased the AUC modestly, by 0.011 for hs-CRP alone, 0.020 for Lp-PLA2 alone, and 0.042 whe
PLA2, and its interaction term were added. The investigators reported that, with the use of traditional risk factors t
risk for ischemic stroke, 86 % of participants were categorized as low-risk (less than 2 %); 11 %, intermediate-risk
3 %, high-risk (greater than 5 %). The addition of hs-CRP, Lp-PLA2, and their interaction to the model re-classified
34 % of the low-, intermediate- and high-risk categories, respectively. The investigators stated that, based on their
addition of both hs-CRP and Lp-PLA2 seems to satisfy the statistical requirements for a test to improve risk predict
investigators stated, however, that the more important question is whether the improvement conferred by the addit
clinically important and cost-effective. The investigators noted that the addition of hs-CRP and Lp-PLA2 did chang
approximately 13 % of the study population. "It would be ideal to validate our findings in other cohorts, conduct stu
changes in therapy secondary to such a risk stratification scheme will improve ischemic stroke prevention, and exa
effectiveness of such a strategy."

Randomized clinical studies of statin therapy for hyperlipidemic persons have shown lower incidence of stroke in t
(Armarenco and Labreuche, 2009); prospective randomized studies of statins for prevention of recurrence in stroke
shown marginal effects (Manktelow and Potter, 2009). However, it is not known whether treatment with statins wo
risk in a subset of normo-lipidemic patients for whom statin therapy would otherwise not be indicated. In addition,
studies have also shown that certain drugs can have an impact on Lp-PLA2 levels; these studies, however, do not
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
improves clinical outcomes. ATPIII guidelines do not include a recommendation for Lp-PLA2 testing in assessme
Guidelines from the American Heart Association and the American Stroke Association (Goldstein et al, 2006) on pr
of ischemic stroke state: "No recommendations about Lp-PLA2 modification can be made because of an absence
showing clinical benefit with reduction in its blood levels." A consensus statement from the American College of C
American Diabetes Association on management of patients with cardiometabolic risk makes no mention of Lp-PLA
American Association of Clinical Chemistry (AACC, 2009) has stated that Lp-PLA2 is not widely availa
findings from recent studies support the potential usefulness of Lp-PLA2 in CHD and ischemic stroke risk assessm
clinical utility has yet to be established." Canadian Cardiovascular Society guidelines (Genest, et al., 2009) do not
PLA2 for screening for heart disease risk. The American College of Cardiology and the American Heart Associatio
Lp-PLA2 and concluded that it might be reasonable for assessment in intermediate-risk asymptomatic adults. This
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
general population; study limitations or bias are present. The guidelines state that LpPLA2 remains a "second-line"
risk estimation. The guidelines suggest that LpPLA2 may be measured as part of a refined risk assessment in pati
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 cla
recommendation for the use of Lp-PLA2. "Measurement of inflammatory markers such as hs-CRP or Lp-PLA2 in p
CVD may be considered to identify patients who may be at increased risk of stroke, although their effectiveness (ie
routine clinical practice) is not well established."

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

Other guidelines make no recommendation for measurement of Lp-PLA2 (New Zealand Guidelines Group, 2009; N
Disease Prevention Alliance, 2009; Lindsay, et al., 2010; National Vascular Disease Prevention Alliance, 2012). An
Lung and Blood Institute (2012) guideline on cardiovascular disease risk in children and adolescents found insuffi
recommend the measurement of inflammatory markers in youths.
An ad-hoc panel of Lp-PLA2 investigators recommended consensus guidelines for Lp-PLA2 use in clinical practice (2008). The panel recommended Lp-PLA2 testing as an adjunct to traditional risk factors in determining the target treatment in correlation with absolute risk. The panel did not recommend Lp-PLA2 testing as a screening tool for low absolute risk. 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 tool in clinical management.

Bertoia et al (2013) examined the prospective association between oxidation-specific biomarkers, primarily oxidize (OxPL) on apolipoprotein B-100-containing lipoproteins (OxPL/apoB) and lipoprotein (a) [Lp(a)], and risk of PAD. They 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 consisted of 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% confidence interval (CI) 1.21-1.55, 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 1.10-1.71, for each 1-SD increase). Autoantibodies to MDA-LDL and ApoB-IC were not consistently associated with risk of PAD in men and women. The major lipid biomarker, OxPL, Lp(a), was also associated with risk of PAD, reinforcing the key role of OxPL in the pathophysiology of atherothrombosis.

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

Carotid intima-media thickness:

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

ATPIII reports that the extent of carotid atherosclerosis correlates positively with the severity of coronary atherosclerosis. 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 been determined in prospective studies. ATPIII concluded that "its expense, lack of availability, and difficulties in implementation preclude a current recommendation for its use in routine risk assessment for the purpose of modifying intensity of L therapy."

A consensus statement from the ADA and the ACC observed that measurements of carotid intima media thickness measurement of coronary calcification and ankle-brachial index, can detect the presence of so-called subclinical atherosclerosis in patients with documented subclinical atherosclerosis are at increased CVD risk and may be considered aggressive therapy. The consensus statement concluded, however, that it is unclear whether such tests improve 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 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 measure
be performed routinely, but may be used in certain clinical situations as adjuncts to standard CVD risk factors in an
risk stratification and the need for more aggressive preventive strategies. This is a grade 4 recommendation, based
level evidence).

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

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

Measurement of arterial elasticity:

Arterial elasticity has been shown to decrease with aging and with vascular disease. A number of studies have de
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
vascular events (Mattace-Raso et al, 2006; Willum-Hansen et al, 2006). In the Rotterdam Study, the adjuste
coronary disease or stroke in the 2nd and 3rd tertiles was 1.72 and 2.45 compared to the lowest tertile (Mattace-R.
The predictive value was independent of cardiovascular risk factors, carotid intima-media thickness, and pulse pres
carotid artery distensibility was not independently associated with CVD.

Hypertension Diagnostics, Inc. (HDI, Eagan, MN) has developed a method of analyzing blood pressure waveforms
measure the elasticity (compliance) of arteries and arterioles. The HDI CVProfiler and the HDI PulseWave graphs
waveform ("pulse contour analysis") and calculates the elasticity (flexibility) of large and small arteries and arteriole
obtains blood pressure and waveform data by use of a blood pressure cuff placed on the left upper-arm and a piez
direct contact, acoustical transducer placed over the right radial artery near the wrist. A computer performs a pulse
of blood pressure waveform data, and generates a report which includes a large artery elasticity index (a measure
compliance) and a small artery elasticity index (a measurement of oscillatory or reflective compliance). The CVPro
measurements of standard blood pressure values (systolic, diastolic and mean arterial pressure), heart rate, body s
and BMI. Arterial elasticity has been investigated as an early marker of vascular disease in patients without stand
CVD. Several studies have examined the impact of various factors on arterial elasticity, and have examined the qu
arterial elasticity is an independent risk factor for cardiovascular disease. However, there is inadequate evidence f
clinical studies demonstrating that non-invasive measurements of arterial elasticity using the CVProfiler alters patie
and improves clinical outcomes. Current guidelines from leading medical professional organizations do not includ
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 p
by HDI/PulseWave CR-2000), and systemic arterial compliance show poor agreement with central pulse wave vel
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 inflam
and the IL-6 -174 g/c promoter polymorphism appears to influence IL-6 levels. Previous findings on the relation be
polymorphism and risk of CVD are inconsistent. Sie and colleagues (2006) examined this polymorphism in relation
population-based study and meta-analysis. Subjects (n = 6,434) of the Rotterdam Study were genotyped. Anal y
between genotype and CHD were performed using Cox proportional hazards tests, and the association between ge
plasma levels of IL-6 and CRP was investigated. All of the analyses were adjusted for age, sex, and common card
factors. A meta-analysis was performed, using a random effects model. No association between genotype and risk observed. The polymorphism was not associated with IL-6 levels, but the C-allele was associated with higher CRP. 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 (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):

Higher levels of the leukocyte enzyme myeloperoxidase (MPO), which is secreted during acute inflammation and precipitation of lipoproteins, are associated with the presence of coronary disease (Zheng et al, 2001; Zheng et al, 2004) and an acute coronary syndrome in patients with chest pain (Brennan et al, 2003). Stefanescu et al (2008) found that patients with CAD had increased CVD risk if plasma MPO levels were elevated and a small study demonstrated that MPO deficiency was associated with increased risk of CHD against CVD (Kutter and Devaquet, 2000). Furthermore, among patients with chronic systolic heart failure (HF), elevated MPO levels have been associated with an increased likelihood of more advanced HF and may be predictive of a higher risk 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 levels have been shown to be predictive of a future myocardial infarction (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).

Apolipoprotein A-1

Apolipoprotein A1 (Apo A1) is the major protein constituent of HDL cholesterol. Although most guidelines recommend risk assessment based on LDL, measurement of Apo A1 has not been established as a clinically useful test at this time. However, some studies have found that Apo A1 predicts cardiovascular disease, it has no more predictive value than more available markers, such as the non-HDL cholesterol level and the ratio of total to HDL cholesterol. In a secondary prospective cohort study involving 15,632 healthy women in the Women's Health Study, investigators assessed the predictive value of various markers. Subjects were followed for at least 10 years, during which time 464 had first cardiovascular events (MI, ischaemic heart disease, or death). After adjustment for age, smoking status, blood pressure, diabetes, and BMI, plasma MPO levels were significantly associated with the risk of developing a cardiovascular event in the most extreme quintiles for each marker (compared with the most favorable quintile). The hazard ratios were as follows: LDL cholesterol level, 1.62; apolipoprotein A-I level, 1.75; total cholesterol level, 2.08; HDL cholesterol level, 2.50; non-HDL cholesterol level, 2.51; CRP level, 2.98. For lipid ratios, the hazard ratios were: LDL:HDL cholesterol, 3.01; LDL:apo A-I, 3.18; apo B:apo A-I, 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, added little to existing measures of risk assessment (van der Steeg et al, 2007). United Kingdom researchers evaluated the ratio of apolipoprotein B to apolipoprotein A-I was associated with CAD among 869 adults with CAD and 1,511 controls, adjusted for age, sex, and time of enrollment. The highest quartile of the apolipoprotein ratio was significantly associated with CAD (odds ratio, 1.85) in analyses adjusted for cardiovascular risk factors (sex, diabetes, BMI, smoking, systolic blood pressure, and LDL and HDL cholesterol levels). The ratio also was associated with CAD (odds ratio [OR], 1.77) in an analysis restricted to the Framingham risk score (a well-established algorithm for combining risk factors to predict CAD). However, the ratio of total cholesterol to the apolipoprotein ratio categorized cases and controls similarly. In addition, the proportion of patients who were predicted to have higher risk for CAD was similar when both ratios were used and when the apolipoprotein ratio was used to the Framingham risk score. An editorialist commented that "risk factor proliferation puts patients and clinicians in a difficult position" (Berkwits and Guallar, 2007).
A report from the Framingham Offspring Study, a large, population-based, cohort study, found that apo A-1 ratio had utility in predicting incident coronary heart disease, and that measuring total cholesterol and HDL appears to suffice heart disease risk (Ingelsson et al, 2007). More than 3,300 middle-aged, white participants in the Framingham Offspring Study were followed for a median of 15 years. A total of 291 first CHD events occurred, 198 of them in men. Elevations in non-HDL cholesterol, apo B, total cholesterol:HDL ratio, LDL:HDL ratio, and apo B:apo A-1 ratio were associated with increased CHD risk to a similar degree. Elevated apo A-1 and HDL were likewise associated with increased CHD risk in women, perhaps owing to the lack of statistical power of these substudies. In men, total cholesterol:HDL and apo B:apo A-1 both improved re-classification of 10-year risk for CHD; however, the difference between the two was not significant. 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 as individual components, and apolipoproteins were better predictors than their cholesterol counterparts. The APO A was the strongest predictor, with a population-attributable risk of 54%, compared with risks of 37% for LDL/HDL and 17% for cholesterol/HDL. A 1-standard-deviation increase in Apo B/Apo A1 was associated with an odds ratio of 1.59 for MI and 1.17 for an equivalent increase in total cholesterol/HDL. The results were similar for both sexes and across all ethnicities. 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.

A meta-analysis found no relationship between apo A1 and apo B and stroke risk (Emerging Risk Factors Collaborative). An individual-patient meta-analysis, aimed at providing clear estimates of the vascular risks associated with lipid levels, was performed on data from 302,430 people without vascular disease at baseline; of these, 32 studies provide stroke outcomes in more than 173,000 people. Non-HDL cholesterol level was modestly associated with ischemic stroke, triglyceride and HDL cholesterol levels were not associated with either ischemic or hemorrhagic stroke risk. Both non-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 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 that Apo A1 is the major apoprotein of HDL. The consensus stated that the apoB:apoA1 ratio is one of the strongest risk markers. The guidelines note, however, that it is still unclear whether this variable should be used as a treatment goal. "As the measurement of apolipoproteins is not available in Europe, is more costly than currently used lipid variables, and does not add more information, its use is not as yet recommended."

Peripheral arterial tonometry

Endothelium plays an important role in the maintenance of vascular homeostasis. Nitric oxide (NO) is the key mediator; it is a potent vasodilator, it inhibits platelet aggregation, vascular smooth muscle cell migration and pro-inflammatory cytokines. Cardiovascular risk factors promote development of endothelial dysfunction, characterized by 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 evaluated by measurement of the vessel response to endothelial agonists, such as acetylcholine (gold standard). The technique for the detection of EDV employs the ultrasound evaluation of flow-mediated dilation (FMD) of the brachial artery.
reactive hyperemia. A close relation between FMD and coronary vasomotor response to acetylcholine has been re
Endothelial dysfunction in the coronary circulation may precede development of angiographically evident coronary
endothelial dysfunction has been also associated with a higher prevalence of CAD and resulted predictive of future
events; recently, it has been associated with a higher risk of re-stenosis after coronary stent implantation. Endothe
actually considered a reversible phenomenon; drug therapies with angiotensin converting enzyme (ACE) inhibitors
receptor blockers, statins, anti-oxidants agents have shown a beneficial effect on endothelial function (Patti et al, 2
Peripheral arterial tonometry (PAT) has been proposed as a non-invasive method to measure endothelial dysfunct
identify patients with early-stage CAD. Endothelial dysfunction is measured by the PAT signal that is obtained usi
PAT2000 device (Itamar Medical) and proprietary software. The test involves the measurement of blood flow in the
following compression of the upper arm with an inflatable cuff. The Endo-PA2000 was cleared by the FDA through
process in November 2003. It is indicated for use as a diagnostic aid in the detection of coronary artery endothelia
(positive or negative) using a reactive hyperemia procedure. The device is not intended for use as a screening tes
patient population. However, there is currently insufficient evidence to support the use of PAT in assessing CAD ri
Kuvin et al (2007) assessed endothelial function in 2 peripheral vascular beds before and during reactive hyperemi
clinic setting. The brachial artery was imaged with a portable ultrasound device and changes in vessel diameter w
"% FMD". Pulse wave amplitude of the finger was detected by PAT and PAT hyperemia was defined as the maxim
plethysmographic recording compared to baseline. A total of 60 individuals (43 men) were enrolled with an averag
years (mean +/- SE). The 31 individuals with more than 2 cardiac risk factors (CRF) had lower FMD (7.0 +/- 1.1 %
hyperemia (2.1 +/- 0.9) compared to the 29 persons with 0 to 2 CRF (FMD 11.3 +/- 0.8 %, PAT hyperemia 2.4 +/-
both). The 32 individuals with CAD had lower FMD (6.8 +/- 1.1 %) and PAT hyperemia (2.0 +/- 0.1) compared to th
without CAD (FMD 11.5 +/- 0.8 %, PAT hyperemia 2.4 +/- 0.1; p < 0.05 for both). Thus, peripheral vascular endotho
testing in the ambulatory setting correlates with the extent of CAD risk and the presence or absence of CAD. The a
that these data suggested that peripheral vascular endothelial function testing is feasible in ambulatory patients, a
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 d
endothelium is an early marker of the development of atherosclerotic changes and can also contribute to cardiovas
Vascular reactivity tests represent the most widely used methods in the clinical assessment of endothelial function
decades, several methodologies were developed to study it non-invasively in the peripheral macro-circulation (cond
micro-circulation (resistance arteries and arterioles). These investigators reviewed the most relevant available non
techniques in the research on endothelial function, their advantages and limitations. Flow mediated dilation of the
ultrasounds is the most widely used vascular test to ascertain endothelium-dependent vasodilation. Other approac
measurement of micro-circulatory reactive hyperemia by fore-arm venous plethysmography or digital pulse amplitu
response to beta-2 agonist by applanation tonometry or digital photo-plethysmography and several test by skin lase
appears that FMD is the most reproducible test when an appropriate and accurate methodology is applied. Syste
proposed as measures of NO biology, inflammatory cytokines, adhesion molecules, or markers of endothelial dam
have only a very limited role as a result of biological and assay availability and variability, these factors currently h
the assessment of individual patients. The optimal methodology for investigating the multi-faceted aspects of endo
is still under debate. Thus, no available test to assess endothelial function has sufficient sensitivity and specificit
clinical practice. Only the growing concordant results from different reproducible and reliable non-invasive method
endothelial function with different stimuli will support and strengthen experimental findings, thus providing conclusiv
area of research.
Chemla and associates (2008) reviewed recent advances in the non-invasive assessment of arterial pressure (indi
the field of critical care. Automated oscillographic measurements under-estimate intra-arterial systolic blood pressu
plethysmography has led to conflicting results, although the obtained respiratory pulse pressure variation correlates
challenge-induced changes in stroke volume. The pulse oximetry photo-plethysmographic signal recorded at the d
may be useful in monitoring respiratory arterial pressure variations, although technical improvements and clarificat
Arterial tonometry is increasingly used in the cardiovascular field to reconstruct central aortic pressure. A recent st
that radial artery tonometry is feasible in hemodynamically stable patients and that peripheral pulse pressure reflects influences of arterial stiffness and stroke volume, especially in elderly patients. The limitations of this technique in 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 from 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 is a risk factor for adverse cardiac events. These researchers tested an easily administered, non-invasive technology to identify visual 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 active periods. Heart rate and blood pressure were assessed, and blood was drawn for catecholamine assay, during rest and active periods. Myocardial ischemia provoked by emotional stress was defined by the presence of a new perfusion defect during activity (n = 26) and the ratio of stress to rest PAT response was calculated. Patients with MSI had a significantly lower PAT ratio without MSI (0.76 +/- 0.04 versus 0.91 +/- 0.05, p = 0.03). An ROC curve for optimum sensitivity/specificity of PAT of MSI produced a sensitivity of 0.62 and a specificity of 0.63. Among patients taking ACE inhibitors, the sensitivity of the test increased to 0.86 and 0.73, respectively; 90% of patients without MSI were correctly identified. 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 from non-invasive techniques, amongst which arterial tonometry seems promising.

**B-type natriuretic peptides:**

In a systematic review and meta-analysis on B-type natriuretic peptides (BNP) and cardiovascular risk, Di Angelan 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 this marker in general populations or in patients with stable vascular disease is uncertain. These investigators collated data from prospective studies involving a total of 87,474 participants and 10,625 incident CVD outcomes. In a comparison of the top-third with those in the bottom-third of baseline values of natriuretic peptides, the combined RR, adjusted for conventional risk factors, was 2.82 (95% CI: 2.40 to 3.33) for CVD. Analysis of the 6 studies with at least 250 CV events should be less prone to selective reporting than are smaller studies) yielded an adjusted RR of 1.94 (95% CI: 1.57 to 2.42) and by different baseline vascular risk (RR, 2.68 [95% CI: 2.07 to 3.47] in approximately general populations; RR, 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 CAD. BNP or NT-proBNP in addition to measurement of conventional CVD risk factors yielded generally modest improvement in discrimination. The authors concluded that available prospective studies indicate strong associations between circulatory 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 understand the relationship between these markers and CVD risk.

Melander and co-workers (2009) assessed the utility of contemporary biomarkers for predicting cardiovascular risk and conventional risk factors. A total of 5,067 participants (mean age of 58 years; 60% women) without CVD were included in the study. Participants underwent measurement of CRP, cystatin C, Lp-PLA2, mid-regional pro-adrenomedullin (MR-proADM), atrial natriuretic peptide, and N-terminal pro-B-type natriuretic peptide (N-BNP) and underwent follow-up using the hospital discharge and cause-of-death registers and the Stroke in Malmo register for first cardiovascular events (e.g., coronary death). Main outcome measures were incident cardiovascular and coronary events. During median follow-up there were 418 cardiovascular and 230 coronary events. Models with conventional risk factors had C statistics of 0.734 to 0.781 and 0.761 (0.730 to 0.789) for cardiovascular and coronary events, respectively. Biomarkers retained elimination models were CRP and N-BNP for cardiovascular events and MR-proADM and N-BNP for coronary events. The proportion of participants reclassified into lower risk categories was modest (8% for cardiovascular risk, 5% for coronary risk). Net reclassification improvement was not statistically 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 improvement...
observed in analyses restricted to intermediate-risk individuals (cardiovascular events: 7.4 %; 95 % CI: 0.7 % to 14 coronary events: 14.6 %; 95 % CI: 5.0 % to 24.2 %; p = 0.003). However, correct re-classification was almost enti down-classification of individuals without events rather than up-classification of those with events. The authors con selected biomarkers may be used to predict future cardiovascular events, but the gains over conventional risk fact Risk classification improved in intermediate-risk individuals, mainly through the identification of those unlikely to de They stated that "[t]hese data do not exclude a future role for circulating biomarkers as adjuncts to conventional ris they minimize the potential for biomarkers to provide insight into underlying mechanisms of diseases. Several biom lead to shifts in predictive accuracy that were at least statistically significant. The challenge will be to find new card biomarkers that alone or in combination with existing biomarkers can bring about improvements in risk assessment statistically but clinically significant as well". Commenting on this study, Schwenk (2009) concluded that this study several markers that are associated with CAD and other cardiovascular diseases in high-risk populations do not pr incremental predictive value over known demographic and clinical risk factors in low-to-moderate risk community-b For now, more-precise personalized approaches to risk stratification and subsequent prevention of cardiovascular available."

An assessment by the National Academy of Clinical Biochemistry (Christenson et al, 2009) stated that measureme natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP) concentrations for CVD risk assessment in the prima setting is unwarranted. Similarly, guidelines from the American College of Cardiology and the American Heart Ass 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 deriv peptide of B-type natriuretic peptide (BNPsp) not only is detectable in cytosolic extracts of explant human heart tis secreted from the heart into the circulation of healthy individuals. Furthermore, plasma levels of BNPsp in patients acute ST-elevation myocardial infarction (n = 25) rise to peak values (about 3 times higher than the 99th percentile range) significantly earlier than the currently used biomarkers myoglobin, creatine kinase-MB, and troponin. Prelim operating characteristic curve analysis comparing BNPsp concentrations in ST-elevation MI patients and other pati positive (AUC = 0.97; p < 0.001), suggesting that further, more rigorous studies in heterogeneous chest pain patien warranted. The authors concluded that these findings demonstrated for the first time that BNPsp exists as a distin human circulation and could serve as a new class of circulating biomarker with the potential to accelerate the clinic cardiac ischemia and myocardial infarction.

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

Mid-regional pro-atrial natriuretic peptide:

The rapid and reliable estimation of prognosis in acute ischemic stroke is pivotal to optimize clinical care. Mid-regi natriuretic peptide (MR-proANP), a recently described, stable fragment of the ANP precursor hormone, may be use In a prospective observational study, Katan and colleagues (2010) examined the prognostic value of MR-proANP acute ischemic stroke. These researchers measured MR-proANP on admission in plasma of 362 consecutive patie acute ischemic stroke. The prognostic value of MR-proANP to predict mortality within 90 days and functional ouc modified Rankin Scale of less than or equal to 2 or greater than or equal to 3) was evaluated and compared with th Institutes of Health Stroke Scale (NIHSS) score. The discriminatory accuracy, calculated with the AUC of the rece characteristics curve, of MR-proANP to predict death was comparable to the NIHSS (AUC: 0.86 [95 % CI: 0.82 to % 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] -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 The prognostic value of MR-proANP for both outcomes was independent of the NIHSS. Higher MR-proANP conce found in stroke of cardioembolic etiology. The authors concluded that MR-proANP is a prognostic marker in the ac stroke, improving the discriminatory value of the NIHSS, independently predicting post-stroke mortality and functio
In an editorial that accompanied the paper by Katan et al, Granger and Laskowitz (2010) stated that the current study at a single center with only 44 deaths, and the results need to be validated in an independent study. A number of questions remain. Does this biomarker change predicted risk enough to alter recommended therapy? Does use of the biomarker improve care and clinical outcomes? And is it cost-effective?

Guidelines from the American College of Cardiology/American Heart Association (2010) and the National Academy of Biochemistry (2009) do not recommend measurement of natriuretic peptides for CVD risk assessment in asymptomatic individuals at risk for or patients with CHD. Large RCTs are needed to ascertain the clinical value of these biomarkers in the management of CHD.

Measurement of long-chain omega-3 fatty acids in red blood cell membranes:

Higher palmitic and lower long-chain omega-3 fatty acids (e.g., alpha-linolenic, eicosapentaenoic and docosahexaenoic) are correlated with higher incidence of CHD in middle-aged men at high risk for CVD (Simon et al, 1995). Immune plasma fatty acids and vitamins E and C were the only factors found related to improvements in life expectancy an 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 recommended for those without and known CHD, respectively. Based on animal and isolated cell studies, the presumed to have anti-arrhythmic effects. It has been proposed that red blood cell (RBC) fatty acids composition, of long-term intake of eicosapentaenoic plus docosahexaenoic acids, can be considered a new, modifiable, and clinical factor for death from CHD.

However, there is a lack of scientific evidence regarding how measurements of RBC omega-3 fatty acids contribute to lipid core growth. Studies have suggested that cholesterol transported by erythrocytes and deposited into the necrotic core of atheroma contributes to lipid core growth. Tziakas and colleagues (2007) hypothesized that cholesterol content is increased in erythrocytes of patients with ACS and may be a marker of clinical instability. Thus, these researchers investigated differences in erythrocyte membranes of patients presenting with ACS compared to patients with chronic stable angina. Consecutive angina patients were prospectively assessed; 120 had CSA (83 men, age of 64 +/- 11 years) and 92 ACS patients. Total cholesterol content in erythrocyte membranes (CEM) was measured using an enzymatic content assay. The CEM (median and inter-quartile range) was higher (p < 0.001) (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 110.5 microg/mg) (analysis of co-variance). Total plasma cholesterol concentrations did not correlate with CEM levels (r = 0.628). The authors concluded that these findings showed for the first time that CEM is significantly higher in patients compared with CSA patients. They suggested a potential role of CEM as a marker of atheromatous plaque growth.

The authors stated that further studies are needed to elucidate the role of CEM as both a marker of plaque instability and pathogenic mechanism of rapid CAD progression. In an editorial that accompanied the above-mentioned article, Arbus et al., although widely investigated either as total cholesterol content or phospholipid/cholesterol ratio, CEM did not have clinical applications”.

Tziakas and associates (2009) evaluated the effect of statin therapy on CEM levels (a novel marker of CAD instability) year follow-up in CAD patients. A total of 212 consecutive eligible patients (158 men, mean age of 62 +/- 10 years) diagnostic coronary angiography for the assessment of angina pectoris were assessed. The study population consisted of 128 ACS patients. All study participants were commenced on statin treatment in equipotent doses an up to 1 year (at 1, 3, 6 and 12 months). Repeated measurements analysis of variance after appropriate adjustment for significant decrease (p < 0.001) in CEM content during follow-up. Levels of CEM were decreasing at each time point. Table 1 shows the results of the study.

**Table 1. Changes in CEM Content during Statin Treatment**

<table>
<thead>
<tr>
<th>Time</th>
<th>CEM Content (microg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission</td>
<td>112.1 (95% CI: 105.9 to 118.3)</td>
</tr>
<tr>
<td>1 year</td>
<td>45.3 (95% CI: 42.2 to 48.3)</td>
</tr>
<tr>
<td>6 months</td>
<td>67.2 (95% CI: 62.0 to 72.4)</td>
</tr>
<tr>
<td>12 months</td>
<td>78.1 (95% CI: 73.2 to 83.0)</td>
</tr>
</tbody>
</table>

The authors concluded that these findings showed for the first time that CEM is significantly higher in patients compared with CSA patients. They suggested a potential role of CEM as a marker of atheromatous plaque growth.
measurements. The authors concluded that these findings showed that the use of statins is associated with a reduced emerging marker of clinical instability and plaque vulnerability in CAD patients. The pleiotropic effects of statins at level represent a promising novel direction for research in CAD.

9p21 and other genetic tests:

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

Guidelines from the Canadian Cardiovascular Society (2009) state that genetic testing for severe lipoprotein disord a few highly specialized centres. The guidelines state, however, that a molecular genetic diagnosis is not necessar patients with severe dyslipidemia; the biochemical and clinical data usually suffice to make a diagnosis. As a resea the molecular study of extreme lipoprotein disorders has provided considerable scientific insight including the ident future therapeutic targets.

European consensus guidelines (2012) make a strong recommendation that DNA-based tests for common genetic not presently add significantly to diagnosis, risk prediction, or patient management and cannot be recommended. T make a strong recommendation that the added value of genotyping, as an alternative or in addition to phenotyping management of risk and early prevention in relatives, cannot be recommended.
A review of genomics of cardiovascular disease published in the *New England Journal of Medicine* (O'Donnell & N concluded: "Genetic risk prediction is at an early stage, and insufficient evidence exists at present to warrant the us score on the basis of SNPs identified through genomic approaches. Additional research is needed to prospectively of genetic risk scores in the prediction of cardiovascular disease, such as myocardial infarction and coronary artery clinical use."

A statement from the American Heart Association (Ashley, et al., 2012) on genetics and cardiovascular disease st robust [genome wide association studies] evidence exists linking common variants to complex CVD, studies are n inform the clinical benefit of providing such genetic information to patients."

Kinesin-like protein 6 (KLP6)

Genome-wide association studies have revealed connections between a number of common single nucleotide pol (SNPs) and cardiovascular diseases. Kinesin-like protein 6 is a protein involved in intra-cellular transport expresse and cell types. A SNP located in the kinesin-like family 6 (KIF6) gene substitutes a thymidine (T) for a cytosine (C) substitution of arginine for tryptophan at amino acid 719 (p.Trp719Arg) of the KIF6 protein. Prospective studies ha carriers of the 719Arg allele in KIF6 are at increased risk of clinical coronary artery disease compared with noncarr been claims that non-carriers of the KIF6 719Arg variant receive little benefit from statin therapy. Screening for this now being used to influence statin use. Celera Corporation (Alameda, CA) has marketed its KIF6 Trp719Arg varia (Statincheck) to cardiologists and primary care physicians. However, a recent study found that statin therapy signi the incidence of coronary and other major vascular events to a similar extent, irrespective of KIF6 genotype (Hopew Investigators sought to test the effects of the KIF6 Trp719Arg polymorphism (rs20455) on vascular risk and respon in 18,348 participants from the Heart Protection Study. Study subjects received 40 mg simvastatin daily for 4 to 6 w randomly allocated 40 mg simvastatin daily or placebo for 5 years. Major coronary event was pre-defined as coro fatal MI, and major vascular event was pre-defined as major coronary event plus re-vascularization or stroke. Th that the KIF6 genotype was not significantly associated, among placebo-allocated participants, with the risks of inc vascular events, major coronary events, re-vascularizations, or strokes. Overall, 40 mg simvastatin daily produced in low-density lipoprotein cholesterol, which did not differ significantly by KIF6 719Arg carrier status (p = 0.51). Pro reductions in the risk of major vascular events with statin therapy were similar (interaction p = 0.70) and highly sign genotypes: 23% (95% CI: 16 % to 29 %; p = 5.3 × 10^{-10}) in carriers (Arg/Arg or Trp/Arg), and 24 % (95 % CI: 17% 10^{-3}) in non-carriers (Trp/Trp). A similar lack of interaction was observed for major coronary events, re-vasculariza considered separately. The authors concluded that the use of KIF6 genotyping to guide statin therapy is not warra therapy significantly reduced the incidence of coronary and other major vascular events to a similar extent, irrespe genotype.

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

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

The CardioVision MS-2000:

The CardioVision MS-2000 is an electronic BP device that calculates an "arterial stiffness index" (ASI) related to falls as the air pressure is released from the BP cuff. Sharma et al (2005) noted that several methods may be used to measure arterial stiffness. One method obtains an ASI from the vascular dynamics of oscillometric-derived brachial artery pressure. Sharma et al (2005) determined the test-retest repeatability of the CardioVision MS-2000. A total of 47 healthy hospital em consecutive measurements of ASI measured after a 5- to 10-min period of rest and then repeated after an average of 323±10 minutes. Their mean age was 37 years and 71% were women. The meanASI was 39.6 +/- 9.7 and 37.2 +/- 10.5 mm Hg x100/mmHg at 1st and 2nd time period, respectively. These researchers computed an intra-class correlation coefficient of 0.31 and 0.35 for the systolic and diastolic BP, respectively. The authors concluded that this study suggested poor test-retest repeatability if measurements are used. The intra-class correlation coefficient, however, could be improved by eliminating the high value from a set of measurements.

According to Barrett (2010), "[a]rterial 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."

Other risk factors:

Non-traditional risk markers have been shown to have statistically significant independent associations with incidence of cardiovascular disease. However, they are not as reliable or cost-effective as standard blood pressure and cholesterol screenings. Using cohort studies, the prospective Atherosclerosis Risk in Communities (ARIC) Study assessed the association of 19 with incident CHD in 15,792 adults followed up since 1987 to 1989 (Folsom et al, 2006). Novel markers included inflammation (CRP, LpPLA2, interleukin 6, D-dimer), endothelial function (intracellular adhesion molecule-1), fibrinogen, fibrin (plasminogen activator inhibitor-1, tissue inhibitor of metalloproteinase-1, soluble thrombomodulin, E-selectin), fibrinolysis (plasminogen, tissue plasminogen activator), B vitamins (leptin, homocysteine, folate, vitamin to infectious agents (Chlamydia IgG positivity, cytomegalovirus antibody, herpes simplex virus-1 antibody). Chang used to assess the additional contribution of novel risk markers to CHD prediction beyond that of traditional risk factor models. Investigators found that the basic risk factor model, which included traditional risk factors (age, race, sex, total and lipoprotein cholesterol levels, systolic blood pressure, anti-hypertensive medication use, smoking status, and diabetes), coronary heart disease well, as evidenced by an AUC of approximately 0.8. The other risk factors did not add significant AUC. Among the novel risk factors, the greatest contribution to AUC was CRP, with an increase in AUC of 0.003. Concluded that routine measurement of these novel markers is not warranted for risk assessment. These findings utility of major, modifiable risk factor assessment to identify individuals at risk for CHD for preventive action. The editorialists explained that these novel markers should not be used for basic risk factor assessment because they 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 is "independent factors is necessary but far from sufficient to demonstrate utility in the prediction of CVD. Rather, predictive utility r demonstration of improvement in test characteristics, predictive values, AUCs (or C statistics), or likelihood ratios a values when a novel marker is added to the existing test. They explained that, from a decision-making point "ultimate" measure of a novel screening test is its ability to reclassify individuals. In other words, a new marker is u 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: Mechan clinical considerations for youth" (Balagopal et al, 2011) listed novel biomarkers for CVD in children including adip peroxisome proliferator-activated receptor, retinol binding protein 4, and resistin. Balagopal et al (2011) also state other products are secreted by adipocytes .... Other discoveries include visfatin, touted to play an important role in glycemic homeostasis, and apelin, the function of which appears to be related to regulation of nutritional intake. Th other adipokines in CVD and T2DM remains unclear. Regarding cytokines, the AHA stated: "Further research veri findings [cytokins such as IL-6 and TNF-α] and better evaluating non-CRP inflammatory processes as they relate to 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 predict stratification improve Framingham risk score (FRS) predictions. A total of 5,933 asymptomatic, community-dwellin (mean age of 69.1 years [SD, 8.5]) were included in this analysis. Traditional CHD risk factors used in the FRS (a blood pressure, treatment of hypertension, total and high-density lipoprotein cholesterol levels, smoking, and diabe CHD risk factors (N-terminal fragment of prohormone B-type natriuretic peptide levels, von Willebrand factor antige levels, chronic kidney disease, leukocyte count, CRP levels, Hcy levels, uric acid levels, coronary artery calcium [C carotid intima-media thickness, peripheral arterial disease, and pulse wave velocity). Adding CAC scores to the F accuracy of risk predictions (c-statistic increase, 0.05 [95 % CI: 0.02 to 0.06]; net re-classification index, 19.3 % ove those at intermediate-risk, by FRS). Levels of N-terminal fragment of prohormone B-type natriuretic peptide also i predictions but to a lesser extent (c-statistic increase, 0.02 [CI: 0.01 to 0.04]; net re-classification index, 7.6 % over at intermediate-risk, by FRS). Improvements in predictions with other newer markers were marginal. The authors among 12 CHD risk markers, improvements in FRS predictions were most statistically and clinically significant with CAC scores. Moreover, they stated that further investigation is needed to assess whether risk refinements using C a meaningful change in clinical outcome.

Guidelines from the American Association of Clinical Endocrinology (2012) do not recommend the routine measure 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 ex association between baseline levels of microRNAs (miRNAs) and incident myocardial infarction (MI) in the Brunec determined their cellular origin. A total of 19 candidate miRNAs were quantified by real-time polymerase chain rea participants. In multi-variable Cox regression analysis, 3 miRNAs were consistently and significantly related to inci showed a positive association (multi-variable HR: 2.69 [95 % CI: 1.45 to 5.01], p = 0.002), whereas miR-223 and m inversely associated with disease risk (multi-variable HR: 0.47 [95 % CI: 0.29 to 0.75], p = 0.002, and 0.56 [95 % C 0.036). To determine their cellular origin, healthy volunteers underwent limb ischemia-reperfusion generated by th and plasma miRNA changes were analyzed at baseline, 10 mins, 1 hr, 5 hrs, 2 days, and 7 days. Computational a temporal clustering by affinity propagation algorithm identified 6 distinct miRNA clusters. One cluster included all m with the risk of future MI. It was characterized by early (1 hr) and sustained activation (7 days) post-ischemia-repe consisted of miRNAs predominantly expressed in platelets. The authors concluded that in subjects with subseqe co-expression patterns of circulating miRNAs occur around endothelium-enriched miR-126, with platelets being a this miRNA signature. The authors stated that these findings await confirmation in independent cohorts. Also, cau inferred from associations of biomarkers in population studies. Furthermore, they stated that future studies will ne 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 despite a considerable size of the study cohort, the total number of incident cases of MI is relatively low, (ii) only a 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 cases of incident MI may have been over-looked. (iii) the present findings await confirmation in an independent cohort before these miRNAs may proceed to make use of these miRNAs as useful tools to enhance stratification for MI.

Jaguszewski et al (2013) noted that Takotsubo cardiomyopathy (TTC) remains a potentially life-threatening disease indistinguishable from acute MI. Currently, no established biomarkers are available for the early diagnosis of TTC from MI. MicroRNAs (miRNAs/miRs) emerge as promising sensitive and specific biomarkers for cardiovascular disease. Investigators sought to identify circulating miRNAs suitable for diagnosis of acute TTC and for distinguishing TTC from 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 (both, n = 36). Consistent with previous publications, cardiac specific miR-1 and miR-133a were up-regulated in TTC patients compared with healthy subjects and up-regulation of miR-16, miR-26a, and let-7f compared with STEMI patients (p < 0.0001, p < 0.05, and p < 0.001). Moreover, miR-133a was substantially increased in patients with STEMI compared with healthy controls (both, p < 0.0001). A unique signature comprising miR-1, miR-16, miR-26a, and miR-133a differentiated TTC from healthy subjects and showed the highest area under the receiver operating characteristic curve (AUC) of 0.835, 95 % CI: 0.733 to 0.937, p < 0.0001. This signature yielded a sensitivity of 74.19 % and a specificity of 78.57 % for TTC versus healthy subjects and a specificity of 70.37 % for TTC versus STEMI patients. Additionally, these researchers noticed a down-regulation of 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 the depression-related miRNAs suggested a close connection of TTC with neuropsychiatric disorders. Moreover, decreased miRNA125a-5p as well as increased plasma levels of its target ET-1 are in line with the microvascular spasm hypothesis of TTC.

Roncarati et al (2014) stated that myocardial miRNAs modulate processes such as cardiomyocyte (CM) hypertrophy, diastolic dysfunction, contractile coupling, and apoptosis; non-CM-specific miRNAs regulate myocardial vascularization and fibrosis. The possibility that circulating miRNAs may be biomarkers of cardiovascular disease has been raised. These research miRNAs (miRNAs) involved in myocardial re-modeling 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 in HCM patients were characterized with conventional transthoracic echocardiography and cardiac magnetic resonance imaging. Plasma levels of 21 miRNAs were assessed by quantitative real-time PCR and were compared with levels in a control group (n = 18) and sex-matched blood donors. Twelve miRNAs (miR-27a, -199a-5p, -26a, -145, -133a, -143, -199a-3p, -29a, -21, and -155) were significantly increased in HCM plasma. However, only 3 miRNAs (miR-199a-5p, -27a, and -29a) correlated with both hypertrophy and fibrosis; more importantly, only miR-29a correlated also with fibrosis. The authors concluded that these findings suggest that cardiac re-modeling with HCM determined a significant release of miRNAs into the bloodstream: the circulating miRNAs hold true for only a few miRNAs (i.e., miR-199a-5p, miR-27a, and miR-29a) correlated with both hypertrophy and fibrosis, identifying it as a potential biomarker for modeling assessment in HCM.

UpToDate reviews on “Screening for coronary heart disease” (Yanowitz, 2013), “Screening for coronary heart disease in diabetes mellitus” (Bax et al, 2013), “Management of proximal left anterior descending coronary artery disease” (Bax et al, 2013) do not mention the use of coronary artery revascularization in HCM. Itabe et al (2011) stated that accumulating evidence indicates that oxidized low-density lipoprotein (OxLDL) is a risk factor for the development of cardiovascular disease. The uptake of OxLDL by scavenger receptors leads to the accumulation of cholesterol and remodelling of atherosclerotic lesions. OxLDL has many stimulatory effects on vascular cells, and the presence of OxLDL in cell membranes...
been established. According to the classical hypothesis, OxLDL accumulates in the atherosclerotic lesions over a leading to advanced lesions. However, recent studies on time-course changes of OxLDL in-vivo raised a possibility be transferred between the lesions and the circulation. These investigators discussed the in-vivo dynamics of OxLDL concluded that recent studies have suggested the plasma OxLDL concentrations may change under pre-pathological conditions. OxLDL may be transferred between tissues and plasma and does not merely accumulate is equilibrated between the tissues and circulation. OxLDL can be formed in various sites in addition to the tissue o
The liver is the major organ for the clearance of OxLDL from circulation. However, many unknowns remain to be whether early recurrent ischemic cerebro were more frequent among carriers of the factor V Leiden or the prothrombin gene mutations than among others. used a case-control design with 367 patients with acute ischemic stroke and atrial fibrillation (cases) and 482 health (controls). All mutations were detected with conventional polymerase-chain reaction protocols. The odds ratios for factor V Leiden, prothrombin gene 20210GA, methylenetetrahydrofolate reductase 677CT, or platelet glycoprotein (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 respectively. Early recurrent ischemic stroke and total recurrent ischemic cerebrovascular events were slightly more among the factor V Leiden mutation than among non-carriers: odds ratio 1.45 (95 % CI: 0.41 to 5.1), and 1.59 (respectively. None of the patients with recurrent ischemic cerebrovascular events had the prothrombin gene mutation concluded that these mutations are not important risk factors for thromboembolic stroke associated with atrial fibrillation the factor V Leiden mutation had a small, non-significantly higher risk of early recurrent ischemic cerebrovascular
Guidelines from the American Stroke Association and the American Heart Association (Goldstein, et al., 2011) stat usefulness of genetic screening to detect inherited hypercoagulable states for prevention of first stroke is not well e he usefulness of specific treatments for primary stroke prevention in asymptomatic patients with hereditary or acqu is not well established."

Also, an UpToDate review on "Overview of the risk equivalents and established risk factors for cardiovascular disease" and "Screening for coronary heart disease" (Yanowitz, 2013) do not mention the use of oxidized LDL triple marker

Berge et al (2007) examined if common prothrombotic mutations are more prevalent in patients with atrial fibrillation 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. used a case-control design with 367 patients with acute ischemic stroke and atrial fibrillation (cases) and 482 health (controls). All mutations were detected with conventional polymerase-chain reaction protocols. The odds ratios for factor V Leiden, prothrombin gene 20210GA, methylenetetrahydrofolate reductase 677CT, or platelet glycoprotein (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 respectively. Early recurrent ischemic stroke and total recurrent ischemic cerebrovascular events were slightly more among the factor V Leiden mutation than among non-carriers: odds ratio 1.45 (95 % CI: 0.41 to 5.1), and 1.59 (respectively. None of the patients with recurrent ischemic cerebrovascular events had the prothrombin gene mutation concluded that these mutations are not important risk factors for thromboembolic stroke associated with atrial fibrillation the factor V Leiden mutation had a small, non-significantly higher risk of early recurrent ischemic cerebrovascular

Bonaca et al (2012) examined if pregnancy-associated plasma protein-A (PAPP-A) is useful for risk assessment in elevation acute coronary syndrome (NSTE-ACS). These investigators measured PAPP-A at baseline in 3,782 pati NSTE-ACS randomized to ranolazine or placebo in the MERLIN-TIMI 36 (Metabolic Efficiency With Ranolazine for Non-ST Elevation Acute Coronary Syndromes) trial and followed for an average of 1 year. A cut-point of 6.0 μIU/m pilot work in this cohort. Pregnancy-associated plasma protein-A greater than 6.0 μIU/ml at presentation was associated 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) an 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 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, individually at 30 days. There was no difference in the risk associated with PAPP-A stratified by baseline cardiac troponin I, ST-segment deviation, age, smoking, hypertension, and CAD, PAPP-A was independently associated with cardiovascular death/MI at 30 days (adjusted HR: 1.35, 95 % CI: 1.07 to 1.71; p = 0.012). Pregnancy plasma protein-A also improved the net re-classification for cardiovascular death/MI (p = 0.003). There was no significant association with ranolazine. The authors concluded that PAPP-A was independently associated with recurrent cardiovascular with NSTE-ACS. They stated that this finding supported PAPP-A as a candidate prognostic marker in patients wit 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 patients within 3 minutes. This FDA-approved, user-friendly, non-invasive device uses a finger probe to observe the change in 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 biomarkers, which are associated with the formation of vulnerable plaque. Vulnerable plaque is responsible for an estimated 25.7% of all heart attacks, so detecting vulnerable plaque is key to determining a patient’s cardiac risk. The test relies on an algorithm applied to 4 clinical risk factors and 7 protein biomarker measurements, to determine who is most at risk of developing unstable angina within a 5-year period. The MIRISK VP test measures serum levels of CTACK, Eotaxin, Fas Ligand, MCP-3, and sFas. These proteins are associated with the biology of vulnerable plaque development. Vulnerable 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 Framingham Risk Assessment. The test offers a significant advancement in CHD risk assessment methods.

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

The majority of first-time coronary angiography patients do not have obstructive CAD. The Corus CAD (CardioDx, peripheral blood gene expression score (GES), consisting of 23 genes, age, and sex, which can assess obstructive 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 for obstructive CAD in nondiabetic patients. Blood samples were obtained prior to angiography in an independent validation set of 526 nondiabetic patients with a clinical indication for coronary angiography. Patients with chronic inflammatory diseases, leukocytes or cardiac protein markers, or diabetes were excluded from the study. Obstructive CAD was defined as coronary artery stenosis in 1 or more major coronary arteries by quantitative coronary angiography. The area under the ROC curve was 0.70 ± 0.02 (P < 0.001); the test added to clinical variables (Diamond-Forrester method) (AUC, 0.72 with the test without; P = 0.003) and added somewhat to an expanded clinical model (AUC, 0.745 with the test vs. 0.732 without). The test improved net reclassification over both the Diamond-Forrester method and the expanded clinical model (P < 0.05) with a threshold that corresponded to a 20% likelihood of obstructive CAD (14.75), the sensitivity and specificity were 85% and 52%, respectively. In a prospective, multicenter study Thomas, et al. (2012) obtained peripheral blood samples for the Corus CAD before perfusion imaging (MPI) in a consecutive series of patients. Patients with abnormal MPI usually underwent invasive coronary angiography, with core laboratories defining coronary artery disease in 431 patients completed GES, coronary imaging (invasive coronary angiography or computed tomographic angiography). Mean age was 56 ± 10 years (48% women). The prespecified primary end point was Corus CAD gene expression receiver-operating characteristics analysis to discriminate ≥ 50% stenosis (15% prevalence by core laboratory and the receiver-operating characteristics curve for the Corus CAD GES was 0.79 (95% confidence interval, 0.73-0.84; sensitivity, specificity, and negative predictive value of 89%, 52%, and 96%, respectively, at a prespecified threshold of patients below this score. The Corus CAD GES outperformed clinical factors by receiver-operating characteristic analysis to discriminate ≥ 50% stenosis (15% prevalence by core laboratory and the receiver-operating characteristics curve for the Corus CAD GES was 0.79 (95% confidence interval, 0.73-0.84; sensitivity, specificity, and negative predictive value of 89%, 52%, and 96%, respectively, at a prespecified threshold of patients below this score. The Corus CAD GES outperformed clinical factors by receiver-operating characteristic analysis to discriminate ≥ 50% stenosis (15% prevalence by core laboratory and the receiver-operating characteristics curve for the Corus CAD GES was 0.79 (95% confidence interval, 0.73-0.84; sensitivity, specificity, and negative predictive value of 89%, 52%, and 96%, respectively, at a prespecified threshold of patients below this score.
reclassification analysis and showed significant correlation with maximum percent stenosis. Six-month follow-up on showed that 27 of 28 patients with adverse cardiovascular events or revascularization had GES >15. Site and core had areas under the curve of 0.59 and 0.63, respectively, significantly less than GES. The investigators concluded CAD GES has high sensitivity and negative predictive value for obstructive coronary artery disease. In this populat 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 p with sable chest pain and related symptoms without a history of CAD were referred to six cardiologists for evaluati prospective cohort of 88 patients, the cardiologist's diagnostic strategy was evaluated before and after gene expres testing. The objective of the study was to measure the effect of the Corus CAD on diagnostic testing using a pre/p There were 83 prospective patients evaluable for study analysis, which included 57 (69%) women, mean age 53 ± mean Corus CAD gene expression score (GES) 12.5 ± 9. Presenting symptoms were classified as typical angina, a and noncardiac chest pain in 33%, 60%, and 7% of patients (n = 27, 50, and 6), respectively. After the Corus CAD diagnostic testing occurred in 58% of patients (n = 48, P < 0.001). The investigators noted that 91% (29/32) of pati decreased testing had low GES (≤ 15), whereas 100% (16/16) of patients with increased testing had elevated GES historical cohort of 83 patients, matched to the prospective cohort by clinical factors, had higher diagnostic test use post-GES prospective cohort (P < 0.001). The investigators concluded that the GES showed clinical utility in the ev 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 diagn The investigators reported on the results of the Primary Care Providers Use of a Gene Expression Test in Coronar Diagnosis (IMPACT-PCP) trial, a prospective study of stable, nonacute, nondiabetic patients presenting with chest symptoms at 4 primary care practices. All patients underwent GES testing, with clinicians documenting their plann strategy both before and after GES. Of the 251 study patients, 140 were women (56%); the participants had a mea (standard deviation, 13.0) and a mean body mass index of 30 mg/kg(2) (standard deviation, 6.7). The mean GES 38), and 127 patients (51%) had a low GES (less than or equal to 15). The investigators noted a change in the diag pattern before and after GES testing in 145 of 251 patients (58% observed vs. 10% predefined expected change; investigators concluded that incorporation of the GES into the diagnostic workup showed clinical utility above and b conventional clinical factors by optimizing the patient's diagnostic evaluation.

Ladapo, et al. (2014) concluded that the results of a registry study demonstrated clinical utility of the Corus CAD b making of primary care providers during assessment of symptomatic patients with suspected obstructive CAD.. The study measured the impact of the Corus CAD Gene Expression Score (GES) on subsequent cardiac referral decis care providers. Of the 342 stable, nonacute patients evaluated, the mean age was 55 years, 53% were female, an was 16 (±10) (range = 1-40). Low GES (≤15), indicating a low current likelihood of obstructive coronary artery disease observed in 49% of patients. The investigators reported that, after clinical covariate adjustment, each 10-point GE associated with a 14-fold decreased odds of cardiac referral (P < .0001). Low GES patients had 94% reduced odds 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 C expression score (GES) for the diagnosis of obstructive CAD. Within a representative commercial health plan's adu current practice for obstructive CAD diagnosis (usual care) was compared to a strategy that incorporates the GES care. The model projected the number of diagnostic tests and procedures performed, the number of patients rece therapy, type I and type II errors for each strategy of obstructive CAD diagnosis, and the associated costs over a 1 Results demonstrate that GES-directed care to exclude the diagnosis of obstructive CAD prior to myocardial perfus yield savings to health plans relative to usual care by reducing utilization of noninvasive and invasive cardiac imag increasing diagnostic yield at ICA. At a 50% capture rate of eligible patients in GES-directed care, it is projected tha health plan will realize savings of $0.77 per member per month; savings increase proportionally to the GES captur concluded that these findings illustrate the potential value of the Corus CAD for health plans and patients in an age 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 concentration of soluble ST2, and has been used to assess prognosis in patients with cardiovascular disease. ST2 the interleukin-1 (IL1) receptor family of cytokines. The manufacturer states that, in the heart, ST2 has a biological immunological processes and is involved in a cardiac signaling pathway, which, under healthy conditions, serves to during pressure overload or stretch. The manufacturer states that ST2 is an emerging biomarker to predict adverse death in individuals with established heart failure and is also a prognostic marker for future cardiovascular disease population.

Published studies of ST2 have focused on its relationship to prognosis in three key areas: 1) risk of hypertension, cardiovascular mortality in the general population (AbouEzziddine, et al., 2012; Wang, et al., 2012); 2) its relationship outcomes in patients with heart failure (Ky, et al., 2011; Felker, et al., 2013); and 3) its prognostic value in acute co (Shrimpo, et al., 2002; Kohli, et al., 2012). However, there is a lack of evidence of clinical utility of ST2 and ST2 has incorporated in current clinical guidelines.

Appendix: Framingham Risk Scoring

Framingham risk scoring for men and women below is adapted from Appendix A of the Executive Summary of the available at the following web site: http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3xsrum.pdf.

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

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

<table>
<thead>
<tr>
<th>Age</th>
<th>Points</th>
</tr>
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<tbody>
<tr>
<td>20-34</td>
<td>-9</td>
</tr>
<tr>
<td>35-39</td>
<td>-4</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>
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<td>8</td>
</tr>
<tr>
<td>60-64</td>
<td>10</td>
</tr>
<tr>
<td>65-69</td>
<td>11</td>
</tr>
<tr>
<td>70-74</td>
<td>12</td>
</tr>
<tr>
<td>75-79</td>
<td>13</td>
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</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>
</table>

http://qawww.aetna.com/cpb/medical/data/300_399/0381_draft.html 01/28/2015
### Cardiovascular Disease Risk Tests

<table>
<thead>
<tr>
<th>Blood Pressure (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>HDL (mg/dL)</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥60</td>
<td>-1</td>
</tr>
<tr>
<td>50-59</td>
<td>0</td>
</tr>
<tr>
<td>40-49</td>
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<tr>
<td>&lt;40</td>
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<table>
<thead>
<tr>
<th>Age Group</th>
<th>Non-smoker</th>
<th>Smoker</th>
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</thead>
<tbody>
<tr>
<td>Age 20-39</td>
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<td>8</td>
</tr>
<tr>
<td>Age 40-49</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Age 50-59</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Age 60-69</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Age 70-79</td>
<td>0</td>
<td>1</td>
</tr>
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<table>
<thead>
<tr>
<th>Systolic BP (mm Hg)</th>
<th>Points</th>
</tr>
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<tbody>
<tr>
<td>&lt;160</td>
<td>0</td>
</tr>
<tr>
<td>160-199</td>
<td>4</td>
</tr>
<tr>
<td>200-239</td>
<td>7</td>
</tr>
<tr>
<td>240-279</td>
<td>9</td>
</tr>
<tr>
<td>≥280</td>
<td>11</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Point Total</th>
<th>10-Year Risk %</th>
</tr>
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<tbody>
<tr>
<td>&lt;0</td>
<td>&lt;1</td>
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<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Age</td>
<td>Points</td>
</tr>
<tr>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>20-34</td>
<td>-7</td>
</tr>
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<td>35-39</td>
<td>-3</td>
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<td>40-44</td>
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</tr>
<tr>
<td>45-49</td>
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</tr>
<tr>
<td>50-54</td>
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<td>55-59</td>
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<tr>
<td>60-64</td>
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</tr>
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<td>65-69</td>
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<td>70-74</td>
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Table 2: Estimated 10-Year Risk for Women (Framingham Point Scores)
<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>≥ 280</td>
<td>13</td>
<td>10</td>
<td>7</td>
<td>4</td>
<td>2</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>HDL (mg/dL)</th>
<th>Points</th>
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<tbody>
<tr>
<td>≥60</td>
<td>-1</td>
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<tr>
<td>50-59</td>
<td>0</td>
</tr>
<tr>
<td>40-49</td>
<td>1</td>
</tr>
<tr>
<td>&lt; 40</td>
<td>2</td>
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</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>Point Total</td>
<td>10-Year Risk %</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>&lt; 9</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1</td>
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</tr>
<tr>
<td>12</td>
<td>1</td>
<td></td>
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<tr>
<td>13</td>
<td>2</td>
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<tr>
<td>14</td>
<td>2</td>
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<tr>
<td>15</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>6</td>
<td></td>
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<td>19</td>
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<tr>
<td>22</td>
<td>17</td>
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<td>23</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>≥ 25</td>
<td>≥30</td>
<td></td>
</tr>
</tbody>
</table>

Guidelines from the American Stroke Association and the American Heart Association (Goldstein, et al., 2011) state commercially available tests exist for the 9p21 and 4q25 risk loci, studies have yet to show that knowledge of genotype leads to an improvement in risk prediction or measurable and cost-effective improvements in patient care.

### CPT Codes / HCPCS Codes / ICD-9 Codes

**High-sensitivity C-reactive protein (hs-CRP): CPT**

codes covered if selection criteria are met:

- 81400 - 81408 Molecular pathology procedures
C-reactive protein; high sensitivity (hsCRP) [2 or more major risk factors, LDL 100-300 intermediate risk of CVD by global risk assessment - see criteria]

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

**ICD-9 codes covered if selection criteria are met:**

- 272.5 Lipoprotein deficiencies [low HDL cholesterol less than 40 mg/dL]
- 305.1 Tobacco use disorder
- 401.0 - 405.99 Hypertensive disease [BP 140 mmHg or higher, or on antihypertensive medication]
- V17.3 Family history of ischemic heart disease [premature CHD]
- V17.49 Family history of other cardiovascular diseases [premature CHD]

**Apolipoprotein B (apo B):**

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

- 82172 Apolipoprotein, each [covered for apoB - not apoA1 or apoE]

**ICD-9 codes covered if selection criteria are met:**

- 250.00 - 250.93 Diabetes mellitus [with 2 or more CVD risk factors - see criteria]
- 272.0 Pure hypercholesterolemia [with 2 or more CVD risk factors - see criteria]
- 272.2 Mixed hyperlipidemia [with 2 or more CVD risk factors - see criteria]
- 305.1 Tobacco use disorder [with 2 or more CVD risk factors - see criteria]
- 401.0 - 405.99 Hypertensive disease [with 2 or more CVD risk factors - see criteria]
- 410.00 - 414.9 Ischemic heart disease [with 2 or more CVD risk factors - see criteria]
- 428.0 - 428.9 Heart failure [with 2 or more CVD risk factors - see criteria]
- 429.2 Cardiovascular disease, unspecified [with 2 or more CVD risk factors - see criteria]
- V17.3 Family history of ischemic heart disease [with 2 or more CVD risk factors - see criteria]
- V17.49 Family history of other cardiovascular diseases [with 2 or more CVD risk factors - see criteria]

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

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

- 0111T Long-chain (C20-22) omega-3 fatty acids in red blood cell (RBC) membranes
- 0126T Common carotid intima-media thickness (IMT) study for evaluation of atherosclerotic coronary heart disease risk factor assessment
- 0311T Non-invasive calculation and analysis of central arterial pressure waveforms with inter report [SphygmoCor System]
<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0337T</td>
<td>Endothelial function assessment, using peripheral vascular response to reactive hyper invasive (eg, brachial artery ultrasound, peripheral artery tonometry), unilateral or bilat</td>
</tr>
<tr>
<td>81240</td>
<td>F2 (prothrombin, coagulation factor II) (eg, hereditary hypercoagulability) gene analysis variant</td>
</tr>
<tr>
<td>81241</td>
<td>F5 (coagulation Factor V) (eg, hereditary hypercoagulability) gene analysis, Leiden va</td>
</tr>
<tr>
<td>81400</td>
<td>Molecular pathology procedure, Level 1(eg, identification of single germline variant [eg techniques such as restriction enzyme digestion or melt curve analysis) [Activated fact plasminogen activator inhibitor (PAIâ€“1)</td>
</tr>
<tr>
<td>81401</td>
<td>Molecular pathology procedure, level 2 (eg, 2-10 snps, 1 methylated variant, or 1 soma [typically using nonsequencing target variant analysis], or detection of a dynamic muta disorder/triplet repeat) [APOE/apolipoprotein E]</td>
</tr>
<tr>
<td>82163</td>
<td>Angiotensin II</td>
</tr>
<tr>
<td>83520</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antig not otherwise specified [adiponectin] [leptin][interleukin-6 (IL-6)] [tumor necrosis factor [Oxidized phospholipids]</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>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 su performed (eg, electrophoresis, ultracentrifugation) [VAP cholesterol test]</td>
</tr>
<tr>
<td>83704</td>
<td>quantitation of lipoprotein particle numbers and lipoprotein particle subclasses (eg, b 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>
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<tr>
<td>84163</td>
<td>Pregnancy-associated plasma protein-A (PAPP-A)</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)</td>
</tr>
<tr>
<td>85384</td>
<td>Fibrinogen; activity</td>
</tr>
</tbody>
</table>
85385  antigen

85414  Fibrinolytic factors and inhibitors; plasminogen activator

88271 - 88275  Molecular cytogenetics [genetic testing] [MIRISK VP test]

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 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)]

93923  Complete bilateral noninvasive physiologic studies of upper or lower extremity arteries (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 single level study with reactive hyperemia) [Digital Pulse Analyzer (DPA)]

99090  Analysis of clinical data stored in computers (eg, ECGs, blood pressure, hematologic data) [CardioVision MS-2000]

Modifier 7A  APOE, commonly called apolipoprotein E (cardiovascular disease or Alzheimer's disease)

Other CPT codes related to the CPB:

93454 - 93461, 93563  Coronary Angiography [coronary artery reactivity test]

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

272.0 - 272.9  Disorders of lipid metabolism

305.1  Tobacco use disorder

401.0 - 405.99  Hypertensive disease

414.00 - 414.07  Coronary atherosclerosis

V15.82  History of tobacco use

V17.3  Family history of ischemic heart disease [premature CHD]

V17.49  Family history of other cardiovascular diseases [premature CHD]

V81.0  Special screening for ischemic heart disease [assessing coronary heart disease risk]
V81.2 Special screening for other and unspecified cardiovascular conditions [assessing coronary heart disease risk]

Homocysteine testing:

CPT codes covered if selection criteria are met:

83090 Homocysteine

ICD-9 codes covered if selection criteria are met:

266.2 Other B-complex deficiencies [vitamin B-12 deficiency]
270.4 Disturbances of sulphur-bearing amino-acid metabolism (e.g., homocystinuria)
415.11 - 415.19 Pulmonary embolism and infarction
453.40 - 453.9 Other venous embolism and thrombosis [unexplained thrombotic disorders]
629.81 Habitual aborter without current pregnancy
634.00 - 634.92 Spontaneous abortion
646.30 - 646.31, 646.33 Habitual aborter
V23.2 Pregnancy with history of abortion
V23.49 Pregnancy with other poor obstetric history [recurrent pregnancy loss]

ICD-9 codes not covered for indications listed in the CPB (not all-inclusive):

V81.0 Special screening for ischemic heart disease [assessing coronary heart disease risk]
V81.2 Special screening for other and unspecified cardiovascular conditions [assessing coronary heart disease risk]

Corus™ CAD gene expression profile:

No specific code

ICD-9 codes covered if selection criteria are met:

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

786.05 Shortness of breath
786.50 Unspecified chest pain
786.51 Precordial pain
786.59 Other chest pain; discomfort, pressure, tightness in chest
413.0 Angina decubitus
413.1 Prinzmetal angina; variant angina pectoris
413.9 Other and unspecified angina pectoris
II. Atypical Anginal Equivalent Symptoms: Require at least one CAD risk factor from III:

724.5 Backache unspecified; acute or chronic pain located in posterior region of thorax, lumb adjacent regions
780.4 Dizziness; light-headedness
780.79 Other malaise and fatigue; lethargy; tiredness
785.1 Palpitations; awareness of heart beat
787.01 Nausea with vomiting
787.02 Nausea alone
787.03 Vomiting alone
787.1 Heartburn
789.00 Abdominal pain

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

272.0 Hypercholesterolemia
272.1 Hyperglyceridemia
272.2 Hyperlipidemia, mixed
272.4 Hyperlipidemia, other and unspecified
277.7 Dysmetabolic syndrome X
278.00 Obesity
277.01 Obesity, morbid
305.1 Tobacco use disorder; tobacco dependence
401.1 Essential hypertension, benign
401.9 Essential hypertension, unspecified
414.01 Coronary atherosclerosis of native coronary artery
433.10 Occlusion and stenosis of carotid artery
437.0 Cerebral atherosclerosis
440.0 Atherosclerosis of the aorta
440.1 Atherosclerosis of the renal artery
440.20 Atherosclerosis of native arteries of the extremities
V15.82 Tobacco use, history
V17.3  Family history of ischemic heart disease
V17.41 Family history of sudden cardiac death

ICD-9 codes not covered for indications listed in the CPB (not all-inclusive):

- 038.0 - 038.9  Systemic infections
- 250.00 - 250.93  Diabetes mellitus
- 410.00 - 410.92  Acute myocardial infarction
- 412  Old myocardial infarction
- 414.00, 414.02 - 414.07  Coronary atherosclerosis
- 428.0  Congestive heart failure, unspecified
- 995.90 - 995.93  Systemic inflammatory conditions
- V45.81  Postprocedural percutaneous transluminal coronary angioplasty status
- V58.65  Postprocedural percutaneous transluminal coronary angioplasty status
- V58.69  Long-term (current) use of other medications [immunosuppressive agents, chemothera

The above policy is based on the following references:

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66U.
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coronary heart disease in Stanford Five-City Project participants. Arterioscler Thromb Vasc Biol. 1997;17(2)
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Toxicol. 1997;81(2):57-64.
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lipoprotein(a). Phase I. Evaluation of the analytical performance of lipoprotein(a) assay systems and comm
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Suppl):S144-S147.
36. Ess SM, Szucs TD. Medical-economical aspects of high sensitivity C-reactive protein assay for the predictio
38. Ockene IS, Matthews CE, Rifai N, et al. Variability and classification accuracy of serial high-sensitivity C-rea
83. Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein particle size to the incidence of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Artery Disease (PLACAD) Study. Am J Cardiol. 2002;90:89-94.


141. (Continued)


145. (Continued)


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210. Manktelow BN, Potter JF. Interventions in the management of serum lipids for preventing stroke recurrence
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Health Dis. 2009;8:41.
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Rockville, MD: Agency for Healthcare Research and Quality (AHRQ); October 2009. Available at:
predicts late cardiovascular adverse events. Eur Heart J. 2010 Feb 24. [Epub ahead of print]
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http://qawww.aetna.com/cpb/medical/data/300_399/0381_draft.html

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292. Yanowitz FG. Screening for coronary heart disease. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed February 2013.


