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Apolipoprotein E (APOE) Genotype, Cardiovascular Risk Test Description

Apo E is a polymorphic glycoprotein attaching to plasma lipoproteins, including chylomicrons, very low density lipoprotein (VLD) cholesterol and HDL-C. Different Apo E isoforms alter plasma lipoprotein concentrations because they have different receptor affinities. This is often henotypically expressed in combination with various environmental stimuli or genetic associations. Apo E has two primary metabolic roles involving its receptor-binding and lipid-binding functions: 1) transport of lipids from their site of synthesis, or absorption, to the tissues where lipids are stored or excreted, and 2) transport of lipids, in particular cholesterol, from the peripheral organs to the liver for excretion. Apo E also modulates the activity of enzymes involved in lipid and lipoprotein metabolism such as hepatic lipase, lipoprotein lipase, cholesterol ester transfer protein and lecithin: cholesterol acyltransferase[1].

The human apo E gene has three common alleles (ε2, ε3, ε4) coding for the Apo E protein as three isoforms (E2, E3, and E4), which vary in the amino acids present at position 112 and 158 of the protein. There are three homozygous (ε4/ε4, ε3/ε3, and ε2/ε2) and three heterozygous (ε4/ε3, ε4/ε2, and ε3/ε2) genotypes and phenotypes, resulting from simple co-dominant Mendelian inheritance of the Apo E gene. The Apo E genotypes include Apo E2 (ε2/ε2, ε2/ε3), Apo E3 (ε3/ε3, ε2/ε4), and Apo E4 (ε3/ε4; ε4/ε4).

Methodology:	Polymerase Chain Reaction/Fluorescence Monitoring
Performed:	Mon - Fri
Reported:	2 days
Specimen Required:	Collect: One 5 mL lavender (EDTA) or pink (K_2EDTA). (Min: 3 mL) Also acceptable: yellow (ACD Solution A), lt. blue (sodium citrate), or green (sodium or lithium heparin). Transport: 5 mL whole blood at 2-8°C. (Min: 3 mL) Unacceptable Conditions: Serum or frozen whole blood. Severely hemolyzed specimens. Stability: Ambient: 3 days; Refrigerated: 1 week; Frozen: Unacceptable
CPT Code(s):	83890 Isolation; 83898 Amplification; 83896 x2 Nucleic acid probes; 83912 Interpretation and report - Additional CPT code modifiers may be required for procedures performed to test for oncologic or inherited disorders.
Reference Interval:	Homozygous apo e3 (e3/e3): This genotype is the most common (normal) genotype.

Clinical Interpretation of Apo E Genotype

Apo E 3/3 is the normal genotype (62% of population) without significant CVD-environment association. Apo E4 (ε4/ε4 and ε4/ε3 - 25% of population) has the highest CVD association related to various clinical atherosclerotic syndromes41-43. These patients are predisposed to an exaggerated elevation of LDL-C when their diet is high in saturated fat. Patients with the Apo E2 genotype tend to have lower LDL-C levels, while Apo E2 and Apo E4 are both associated with elevated triglyceride levels.

In general, Apo E2 and Apo E4 have opposing effects on plasma lipids. Mechanistically, Apo E2 is associated with slow conversion of intermediate density lipoprotein (IDL) to LDL-C leading to a decrease in plasma cholesterol and increased triglycerides. Apo E3 has “normal” lipid metabolism, thus no genotype impact. Apo E4 confers a limitation of HDL-C binding and the normal clearance process is inhibited leading to an increase in total cholesterol, LDL-C and TG.

Apo E binds to lipids, heparan sulfate proteoglycans and lipoprotein receptors (LDL receptor and LDL receptor–related protein); differences in Apo E isoform receptor-binding affect plasma lipid levels. This modulates lipoprotein levels by influencing the clearance rate, lipolytic conversion and triglyceride-rich VLDL production[2]. Apo E2 has markedly decreased LDL-receptor binding affinity as compared with Apo E3, and some reports suggest that Apo E4 has increased affinity as compared with Apo E3[3]. The binding of Apo E to lipoproteins is also isoform dependent with Apo E4 preferentially binding to VLDL and Apo E3 and Apo E2 binding to the smaller, phospholipid-rich HDLC[4]. This complex interaction generally leads to a decrease in LDL-C and HDL-C in Apo E2 compared to Apo E3 and elevated LDL-C and lowered HDL-C in Apo E4[5].

Relevant to lipoprotein subclass determination, it is well recognized that a preponderance of small, dense LDL particles is associated with increased CVD[6,7]. The e4 allele is associated with a decreased LDL particle size and clinical studies show a preponderance of small, dense LDL particles in various classes of Apo E4 patients[4, 8], which will clearly influence a relationship between Apo E4 patients and a higher incidence of CVD.

The Apo E genotype directly influences variations in lipid metabolism and is correlated, under environmental stress, with the phenotypic expression of CVD states consistent with these metabolic differences[9, 1]. Understanding and identifying these gene-environment interactions can influence a therapeutic regimen to better treat dyslipidemias and the associated atherosclerotic cardiovascular disease process. As defined by accepted health guideline standards, therapy targeting these dyslipidemias, a true disease state, results in significant improvements in CVD outcomes.

As a general consideration, we reference the following literature highlighting the Apo E genotype influence on gene-environment interactions and the specific CVD implications for therapeutic options and targeted therapeutic algorithms. The NCEP Guidelines consider lowering LDL-C with statins as a treatment of choice for CVD. Statins, however, are not sufficient therapy in some susceptible individuals due to a wide variability in lipid-lowering drug response, in part related to Apo E genotype influence[10]. This is consistent with the partial CVD event reduction (25-35%) seen in the large statin trials. As example, pertinent to generally accepted considerations for broad-

based statin use to treat elevated LDL-C, subjects carrying the E4 allele are poor responders to lipid-lowering drugs [10, 11, 12], except for probucol[12] and possibly simvastatin[14], in contrast to low fat dietary responsiveness[12].

Current **dietary** recommendations for reducing CVD target atherosclerotic associated diseases such as hypertension, dyslipidemias, obesity and diabetes tend to be broad-based and generic, targeting fat, cholesterol and often sodium restriction. General "guidelines" are applied in a "one-size-fits-all" style for a consensus, at-risk population. In a broad sense, the effectiveness of this approach appears limited, which cannot be explained merely by personal non-adherence to guideline recommendations. An important reason to explain this limitation emerges from studies demonstrating that dietary changes induce a lipid response that varies between individuals (interindividual variability), due to effects of age, gender, ethnicity and genetic heterogeneity, including gene polymorphisms[13]. The American Heart Association's (AHA) Dietary Guidelines (Revision 2000) specifically references the need to consider how underlying genetic and metabolic heterogeneity may limit the potential for generalized nutritional guidelines to address individual dietary responsiveness[15]. In their Scientific Conference proceedings on preventive nutrition, the AHA has also emphasized the need for focusing on the influence of gene polymorphisms on individual responses to dietary factors[16]. This may be particularly relevant for Apo E, recognizing the associated variable lipid-lowering response to dietary fat restriction associated with different Apo E genotypes. Reports note marked variability in the LDL-C lowering response to a restricted fat diet[17-19], which has been particularly ascribed to Apo E gene polymorphisms[20, 21]. Specifically, current recommendations for "standard" cardiac diets are not sufficient in lowering CVD potential in CVD-susceptible, but diet-hyporesponsive, individuals.

Smoking is a major environmental risk factor influencing CVD[22], associated with a 2-fold lifetime CVD risk[23], yet disease develops earlier in some smokers whereas other smokers appear relatively unaffected. An individual's genotype, particularly the Apo E genotype, can affect a variable response to the negative environmental impact of smoking relevant to coronary heart disease and effects on intermediate lipid traits. Apo E4 smokers are reported to have a particularly high CVD risk compared with Apo E4 non-smokers[6, 24]. The additional benefit of smoking cessation is emphasized in that *former* Apo E4 smokers also have a lower CVD risk compared to smokers. Another study analysis showed that compared to Apo E3 individuals, the Apo E4 genotype incurred more than an additive interaction between smoking and genotype with a much higher CVD risk [25.] The more than additive interaction between Apo E4 smokers and CVD was also found in an analysis of the Framingham Offspring data.

Angiographic studies of CHD patients have shown that Apo E4 carriers more often have disseminated and severe coronary lesions than noncarriers[26]. The Apo E genotype is reported to increase an individual carrier's differential susceptibility to CAD events. In larger studies and pooled analyses, Apo E4 is associated with increased CAD events compared to Apo E2 and Apo E3. Reported CAD event risk is increased about 40% in Apo E4 individuals16. A meta-analysis measured clinical events in 6,355 individuals in nine observational studies and found significantly increased total risk for CAD events (Apo E4 compared to Apo E3 - odds ratio 1.26)16. A substudy of the Scandinavian Simvastatin Survival Study (4S) looked at 966 patients with CAD17. Apo E4 subjects who received placebo versus simvastatin had increased risk of CAD-related death (odds ratio - 1.8) compared to non-Apo E4 individuals. A case control analysis of 619 subjects from the Multiple Risk Factor Intervention Trial (MRFIT) found the Apo E4 genotype associated with

increased risk for non-fatal myocardial infarction and CAD-related death, even after adjustment for differences in LDL-C, HDL-C, body mass index, smoking and diastolic blood pressure[27].

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Apo E Literature Summary of Metabolic Responses Seen with Different Apo E Genotypes

		Apo E2		Apo E3		Apo E4	
	Genotype	**2/2**	**2/3**	**3/3**	**2/4**	**3/4**	**4/4**
	Population Frequency	**1%**	**10%**	**62%**	**2%**	**20%**	**5%**
Dietary Contribution	**Soluble Fiber**	⇩ LDL		⇩ LDL		⇩ LDL	
	Fish Oil	⇩⇩ TG ⇩ sd LDL ⇧HDL		⇩⇩ TG ⇩ sd LDL ⇧HDL		⇩⇩ TG ⇩ sd LDL ⇧HDL, may ⇧LDL	
	Plant Sterols	⇩ LDL ⇩ Apo B		⇩ LDL ⇩ Apo B		⇩ LDL ⇩ Apo B	
	Soy Protein	⇩ Apo B		⇩ Apo B		⇩ Apo B	
Dietary Fat & Alcohol Effects	**Low Fat Diet**	⇩ LDL		⇩ LDL		⇩ LDL	
	Moderate Fat Diet	⇔LDL ⇔ sdLDL		⇩ ⇩ LDL ⇩ sdLDL		⇩ ⇩ ⇩ LDL ⇧⇧ sdLDL	
	Moderate Alcohol	⇧ HDL ⇩ LDL		⇧ HDL		⇩ HDL ⇧ LDL	

Legend: ⇩ decreases ⇧ increases ⇔ no change ⇧⇧ significantly increases ⇩⇩ significantly decreases

Apo E Genotype Response Summary

	Apo E Affect on CVD Risk	Proposed Mechanism	Treatment	Response	Benefit
Apo E2 Genotype 2/2 2/3	Intermediate CVD Risk	Slow conversion of IDL to LDL leads to decrease on plasma cholesterol and increase in triglycerides	Statin	⇩ LDL	Beneficial
			Moderate Alcohol	⇩ LDL ⇧ HDL	Beneficial
			Low Fat Diet	⇧ sdLDL Limited ⇩ LDL	Not Recommended
Apo E4 Genotype 3/4 4/4	Highest CVD Risk (42% Increased Risk of CVD)	Diminished binding of HDL leads to increase in LDL and TG; normal clearance process is inhibited	Statin	Limited ⇩ LDL	Limited
			Moderate Alcohol	⇧ LDL ⇩ HDL	Not Recommended
			Low Fat Diet	⇩ LDL ⇩TG ⇩ sdLDL	Beneficial

Legend: ⇩ decreases ⇧ increases

Ann Intern Med 2004 July 20: 141(2):137-47

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Lp-PLA$_2$ Test Description

Lp-PLA2 is a CVD-specific vascular inflammation biomarker which has been shown to be strongly predictive of cardiovascular disease events, including stroke. Medicare has also recognized the clinical utility of Lp-PLA2 in their updated coverage policies for 2007, assigning a unique, dedicated CPT code (CPT 83698) with higher reimbursement.

Methodology:	Enzyme-Linked Immunosorbent Assay
Performed:	Mon- Fri
Reported:	2 days
Specimen Requirements:	Collect: One 4 mL serum separator tube or plasma separator tube. Also acceptable: lavender (EDTA) or pink (K2EDTA). Transport: 1 mL serum or plasma at 2-8 °C. (Min: 0.2 mL) Unacceptable Conditions: Ambient and unprocessed blood samples (EDTA whole blood). Stability: After separation from cells: Ambient: 4 hours; Refrigerated: 1 week.
CPT Code(s):	83698
Reference Interval:	0-234 ng/mL

Clinical Interpretation of Lp-PLA$_2$

Lp-PLA$_2$ is a Predictor of Cardiovascular and Stroke Events :

❶ An independent risk factor for CVD and stroke events [1]
- 2x risk for CVD events when elevated
- 5.5x risk for stroke events when elevated

❷ Predicts CVD and stroke in both elderly men and women [2]

❸ Additive risk with C-Reactive Protein (CRP) [1, 3] or Systolic Blood Pressure (SBP) [3]
- When both Lp-PLA2 and hsCRP are very high — 4x risk for CVD events
- When both Lp-PLA2 and SBP are very high — 6.4x risk for ischemic stroke
- When both Lp-PLA2 and CRP are very high — 11.4x risk for ischemic stroke

❹ Lp-PLA2 with hsCRP predicts reoccurrence and severity of 2nd stroke event [4]

❺ Additive risk from Lp-PLA2 and hsCRP beyond carotid IMT results [5]

❻ Predicts angiographic finding of coronary atherosclerosis [6]

❼ Predicts coronary death [6]

❽ Predicts coronary endothelial dysfunction which is a marker for early atherosclerosis and increased risk of ischemic cardiac events and stroke [7]

Treatment of Lp-PLA$_2$:

- Lp-PLA2 is lowered by statins and fenofibrates [8, 9, 10, 11]
- Niaspan® added to established statin therapy lowers Lp-PLA2 levels by additional 20%[12]
- Lp-PLA2 cut-point values for patients **with known CVD**
- Alert cut-point of > 223 ng/mL [13, 14]
- Goal cut-point of < 200 ng/mL [15]

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Mass Fibrinogen Test Description

Several laboratory methods exist to determine fibrinogen levels. Each method may give results that vary from other methods. Health Diagnostic Laboratory, Inc. uses the immunoturbidimetric method to measure “mass” fibrinogen. This immunoturbidimetric method uses antibodies directed against fibrinogen to detect and quantify levels of fibrinogen. This test offered by HDL is not a functional fibrinogen test. Many other clinical labs use a functional (i.e. coagulation based or clot-based) method to quantify fibrinogen. Results from HDL’s immunoturbidimetric assay by should not be clinically used for evaluation of coagulopathies or bleeding status unless combined with the results of other coagulation (clot-based) laboratory tests.

Methodology:	Immunoturbidimetry
Performed:	Mon- Fri
Reported:	2 days
Specimen Requirements:	Collect: One 4 mL sodium citrate plasma. EDTA up to 500 mg/dl or heparin can also be used. Immediately after collection, centrifuge samples and remove plasma from cells. Transport: 1 plasma at 2-8 °C. (Min: 0.2 mL) Unacceptable Conditions: Ambient and unprocessed blood samples (EDTA whole blood). Stability: 8 hours at room temperature, 2 days at 2-10°C
CPT Code(s):	85384
Reference Interval:	< 350 mg/mL

Fibrinogen is a Predictor of CHD:

Fibrinogen is a plasma glycoprotein which is synthesized by the liver and is involved in the final steps of coagulation when transformed by thrombin into a fibrin clot in response to vascular or tissue injury. Fibrinogen is also a non-specific acute-phase reactant and can be elevated for a variety of chronic inflammatory conditions, including cardiovascular disease. Elevated fibrinogen levels are associated with an increased risk of vascular and non-vascular mortality. The relationship between elevated fibrinogen and disease mortality is not fully understood. A recent meta-analysis[1] demonstrates a moderately strong association between plasma fibrinogen level and the risks of CHD, stroke, other vascular mortality, and nonvascular mortality in a wide range of circumstances in healthy middle-aged adults. In this meta-analysis, the associations of fibrinogen level with CHD or stroke did not differ substantially according to sex, smoking, blood pressure, blood lipid levels, or several features of study design. The hazard ratio ranged from 1.8-2.0 for every 100 mg/dL increase in plasma fibrinogen. This increased risk in mortality was independent of the assay methodology used to measure

fibrinogen (i.e. clot-based vs. non-clot based methods). Inherited disorders reflected by elevated fibrinogen exist that help diagnose the etiology of CAD in individual patients[2]. Fibrinogen levels can be elevated in patients having a genetic predisposition coupled with other environmental factors[3]. These include tobacco use, gender (males routinely have higher values), use of drugs (i.e., some statins, hormone replacement therapy, and oral contraceptives), excess weight, sedentary lifestyle, stress, inflammatory processes (chronic or temporary) and diabetes.

Fibrinogen in association with other cardiac risk markers may produce an additive risk for CVD events[4]. Both Lp(a) and fibrinogen are pro-thrombotic, potentially adding to increased CVD risk when both are elevated[5]. Both CRP and fibrinogen are acute phase reactants and may indicate the presence of an active atherosclerotic process. When both are elevated, the differential diagnosis must eliminate non-cardiovascular, disease-related contributing factors such as acute or chronic inflammatory disorders.

Treatment of Fibrinogen:
Drug therapies that have been reported to reduce fibrinogen include the fibric acid derivatives and nicotinic acid[9, 10].
Factors that lower fibrinogen include smoking cessation, weight loss[6], exercise[7], and alcohol[8].

Clinical References:
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Apolipoprotein B (Apo B) Test Description

Apolipoprotein B (Apo B) is a major protein component of low-density lipoprotein (LDL) comprising >90% of the LDL proteins and constituting 20-25% of the total weight of LDL. Apo B exists in 2 forms. Apo B-100, The most abundant form of Apo B, is found in lipoproteins synthesized by the liver including LDL, VLDL, and IDL. Apo B-48 consists of the N-terminal 2152 amino acids (48%) of Apo B-100, is produced by the gut, and is found primarily in chylomicrons. Apo B- 48 is produced in the intestine, whereas the liver synthesizes Apo B-100. Therefore, lipids derived from exogenous, dietary sources are packaged in lipoproteins (chylomicrons) containing Apo B-48, whereas endogenously produced lipids from the liver are packaged in particles containing apo B-100 (VLDL, IDL, LDL).

Apo B-100 is the major apolipoprotein associated with LDL synthesis in the liver and is a more accurate measurement of the relative number of LDL particles than an LDL-C measurement. Apo B is a true indicator of the number of atherogenic particles. Apo B-100 containing particles are cleared through the LDL receptor, which also recognizes Apo E, but not apo B-48. Higher levels of plasma Apo B may signify increased coronary disease risk even when LDL cholesterol (LDL-C) is not in the high-risk range. For comprehensive cardiac risk assessment, because of the numerical association of Apo-B with the atherogenic VLDL, IDL and LDL particles, it is important to consider the Apo B metric and remember that Apo B is not measured in a standard lipid panel.

Methodology:	Immunoturbidimetry
Performed:	Mon- Fri
Reported:	Within 2 days
Specimen Requirements:	Collect:: One 4 mL serum separator tube. Fasting specimen recommended. Transport:: 1 mL serum at 2-8°C. (Min: 0.5 mL) Submit specimen in an ARUP Standard Transport Tube. Remarks: Separate serum from cells ASAP. Unacceptable Conditions: Hemolyzed specimens. Stability: After separation from cells: Ambient: 8 hours; Refrigerated: 8 days; Frozen: 3 months
CPT Code(s):	82172
Reference Interval:	Male: 55-140 mg/dL, Female: 55-125 mg/dL

Clinical Utility:
It is well established that increased plasma concentration of Apo B-containing lipoproteins is associated with an increased risk of developing atherosclerotic disease. Case control studies have found plasma Apo B concentrations to be more discriminating than other plasma lipids and lipoproteins in identifying patients with coronary heart disease (CHD). The utility of Apo B in determining CHD risk has been confirmed by prospective studies, although the extent to which Apo B concentrations were better than serum lipids in predicting risk was variable. Apo B measurement offers greater precision than LDL cholesterol determination which is most often derived by calculation.

Abetalipoproteinemia and severe hypobetalipoproteinemia can cause malabsorption of food lipids and polyneuropathy. In patients with hyperapobetalipoproteinemia (HALB), a disorder associated with increased risk of developing CHD and with an estimated prevalence of 30% in patients with premature CHD, Apo B is increased disproportionately in relation to LDL cholesterol. Apo B quantitation is required to identify these patients and is necessary in distinguishing HALB from another common lipoprotein abnormality, familial combined hyperlipidemia.

Cautions
Fasting for <12 hours or intake of alcohol during the 24 hours prior to specimen collection may invalidate test results.

Clinical References:

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Lipoprotein (a) [Lp(a)] Test Description

Lipoprotein(a) is a specific heterogeneous class of lipoprotein particles found in human plasma consisting of an apolipoprotein (a) molecule attached to an apolipoprotein B-100 and a lipid-rich LDL-like core. Lp(a) is metabolically distinct from LDL. The protein moiety has two components, a single copy of apolipoprotein B-100 (Apo B-100) linked to a single copy of apolipoprotein(a) [Apo(a)]. The identifiable characteristics of Lp(a), including its size, density and heterogeneity, are almost entirely attributable to the apo(a) protein, which is characterized by marked size heterogeneity. Apo(a) is very similar to plasminogen, a fibrinolytic pro-enzyme, and contains various multiple corresponding plasminogen *kringle* domains. Lp(a) has at least 10 types of plasminogen kringles, explaining the unique and characteristic isoform size heterogeneity. Due to the relationship to plasminogen,

Methodology:	Immunoturbidimetry
Performed:	Mon- Fri
Reported:	Within 2 days
Specimen Requirements:	Collect:: One 4 mL serum separator tube or plasma separator tube. Also acceptable: green (sodium or lithium heparin), lavender (EDTA), or pink (K2EDTA). Remarks: Allow serum to clot completely at room temperature before centrifuging. Separate serum or plasma from cells ASAP. Transport: : 1 mL serum or plasma, frozen. (Min: 0.3 mL) Submit specimen in an ARUP Standard Transport Tube. Unacceptable Conditions: Ambient and unprocessed blood samples (EDTA whole blood). Stability: After separation from cells: Ambient: 8 hours; Refrigerated: 2 weeks; Frozen: 3 months
CPT Code(s):	83695
Reference Interval:	< 30 mg/mL

Clinical Utility:

Lp(a) may have a link between thrombosis and atherosclerosis, interfering with plasminogen function in the fibrinolytic cascade. Numerous studies have documented the relationship of high plasma Lp(a) concentrations to associated with a variety of cardiovascular disorders, including peripheral vascular disease, cerebrovascular disease, and premature coronary disease, although the physiologic and pathologic mechanisms of action have not yet been clearly elucidated. It is assumed that Lp(a) contributes to the atherosclerotic process as it is found at sites of vascular injury, possibly through an interaction in arterial plaque formation.

Lp(a) levels are genetically determined and remain relatively stable over an individual's lifetime. Elevated Lp(a) is an established independent risk factor for cardiovascular events associated with an increased risk for myocardial infarction, stroke, coronary artery disease, vein graft restenosis, and retinal arterial occlusion[1].

Treatment of Lp(a):
Reduction in Lp(a) by hormone replacement therapy or plasma apheresis has been shown to reduce cardiovascular events in post-menopausal women, and limit restenosis following angioplasty[2,3]. Elevated Lp(a) greatly increases cardiovascular event risk in patients with elevated LDL-C or low HDL-C[4,5]. Baseline Lp(a) as an independent risk factor for combined cardiovascular events is reported higher in women and patients with a history of peripheral vascular Clinical disease[6]. Knowledge that Lp(a) is elevated allows the physician to select a therapy that, in addition to other effects, may reduce Lp(a) and consequently, cardiovascular events. These therapies include nicotinic acid and hormone replacement therapy[7].

Clinical References:

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3. Daida H, Lee YJ, Yokoi H et al. Prevention of restenosis after percutaneous transluminal coronary angioplasty by reducing lipoprotein (a) levels with low-density lipoprotein apheresis. Low-Density Lipoprotein Apheresis Angioplasty Restenosis Trial (L-ART) Group. *Am J Cardiol* 1994 June 1;73(15):1037-40.
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7. Superko HR. Hypercholesterolemia and Dyslipidemia. Curr Treat Options Cardiovasc Med 2000 April;2(2):173-87.

Lp(a) Cholesterol Test Description

Lp(a) cholesterol values should not be confused with Lp(a) mass values, although they are highly correlated. Lp(a) cholesterol values will be approximately 10X lower than Lp(a) mass values, but the difference between the measures is not uniform. Lp(a) mass values are considered elevated when >30 mg/dL. Lp(a) cholesterol is increased if > or = 3 mg/dL.

Lipoprotein (a) (Lp[a]) is a highly heterogeneous molecule, consisting of a low-density lipoprotein (LDL) with a highly glycosylated apolipoprotein(a) (apo[a]) covalently linked to the apolipoprotein B moiety of LDL via a single disulfate bond. Lp(a) has been associated with atherogenesis and promotion of thrombosis. Increased levels of Lp(a) have been estimated to confer a 1.5 to 3.0-fold increased risk for coronary artery disease (CAD) in many but not all studies. Apo(a) has approximately 80% structural homology with plasminogen, but does not contain the active site for fibrin cleavage. One proposed mechanism for Lp(a)'s atherogenicity is competition for binding sites with plasminogen during fibrin clot formation and the resulting inhibition of fibrinolysis. Recently a high correlation was demonstrated between Lp(a) and oxidized LDL, suggesting that the atherogenicity of Lp(a) lipoprotein may be mediated in part by associated proinflammatory oxidized phospholipids.

In the clinical laboratory, immunologic methods are generally used to quantify Lp(a) protein mass. Reagents for Lp(a) mass measurement are available from multiple manufacturers and although standardization efforts are underway, currently available methods are not standardized. Difficulties in standardizing Lp(a) mass measurement arise from the variability in signals produced by different reagents due to the size polymorphisms of apo(a). For this reason, some elevations of Lp(a) mass are associated with low levels of Lp(a) cholesterol. Lp(a) quantification can be done by densitometric measurement of Lp(a) cholesterol. This method measures only the cholesterol contained in the Lp(a) particles and is thus not influenced by the relative size of the apo(a) size, it may provide a more specific assessment of cardiovascular risk than Lp(a) mass measurement. Lp(a) cholesterol measurement may be used in concert with Lp(a) mass determination, or may be used as a stand-alone test for assessment of risk.

Methodology:	Electrophoresis, Enzyme Staining, and Densitometry
Performed:	Mon- Fri
Reported:	2 days
Specimen Requirements:	Collect: Draw blood in a plain, red-top tube(s) from a fasting patient (8 hour). Spin down and send 0.4 mL of serum. If sample cannot be shipped immediately, freeze in plastic vial and ship frozen. Transport: 1 serum at 2-8 °C or frozen. (Min: 0.2 mL). Do not ship ambient. Unacceptable Conditions: Ambient and unprocessed blood samples (EDTA whole blood). Stability: 8 hours at room temperature, 2 days at 2-10°C
CPT Code(s):	89005
Reference Interval:	< 3 mg/mL

Clinical Utility:

- Lipoprotein (a) cholesterol is an independent predictor of angiographic coronary artery disease and subsequent cardiovascular events in patients referred for coronary angiography.
- Elevated Lp(a) is a causal factor for MI
- Those with top 10% of Lp(a) levels had a two to threefold higher risk of MI vs. those with lowest Lp(a) levels

Apolipoprotein A1 [Apo A1] Test Description

Apolipoproteins, the protein component of lipoproteins, are complexes that function to transport lipids throughout the bloodstream. Apolipoproteins provide structural integrity to lipoproteins. Most lipoproteins function to transport their cholesterol and triglyceride components for cellular uptake throughout the body. As high density lipoproteins (HDL) circulate systemically, they pick up cellular excess cholesterol for transport to the liver. HDL's reverse cholesterol transport mechanism removes free cellular cholesterol. Upon return to the liver, cholesterol is either excreted into bile or used in new lipoprotein complexes[105].

Methodology:	Immunoturbidimetry
Performed:	Mon- Fri
Reported:	Within 2 days
Specimen Requirements:	Collect:: One 4 mL serum separator tube. Fasting specimen recommended. Transport:: 1 mL serum at 2-8°C. (Min: 0.5 mL) Unacceptable Conditions: Hemolyzed specimens. Stability: After separation from cells: Ambient: 8 hours; Refrigerated: 8 days; Frozen: 3 months
CPT Code(s):	82172
Reference Interval:	Male: 110-180 mg/dL , Female: 110-205 mg/dL
Goal Value:	Male: > 115mg/dL Female: > 140 mg/dL

Clinical Utility:
Apolipoproteins, the protein component of lipoproteins, are complexes that function to transport lipids throughout the bloodstream. Apolipoproteins provide structural integrity to lipoproteins. Most lipoproteins function to transport their cholesterol and triglyceride components for cellular uptake throughout the body. As high density lipoproteins (HDL) circulate systemically, they pick up cellular excess cholesterol for transport to the liver. HDL's reverse cholesterol transport mechanism removes free cellular cholesterol. Upon return to the liver, cholesterol is either excreted into bile or used in new lipoprotein complexes[1].

The Apolipoprotein A (Apo A) family constitute the major proteins found in HDL and triglyceride-rich lipoproteins. Apo A, as part of HDL, is involved in the removal of free cholesterol from extrahepatic tissues and also plays a role in the activation of lecithin acyltransferase. Apolipoprotein A activates the enzymes driving cholesterol transfer from the tissues into HDL and is also involved in HDL recognition and receptors binding in the liver[2]. There are multiple forms of apolipoprotein A, the most common being Apo A1 and Apo A2.

Apo A1 is the major "A" apolipoprotein attached to HDL. Apo A1 is found in greater proportion than Apo A2 (about 3 to 1)[3]. Apo A1 concentration can be measured directly and corresponds with HDL levels. Lower levels of Apo A commonly correlate with the presence of CAD and peripheral vascular disease. Apo A1 may be a better predictor of atherogenic risk than HDL-C[4]. Certain genetic disorders cause Apo A1 deficiencies and associated low levels of HDL. These patients also tend to have hyperlipidemia with elevated LDL. This contributes to accelerated rates of atherosclerosis.

Apo A1 may be increased with:

- Drugs (e.g., carbamazepine, estrogens, ethanol, lovastatin, niacin, oral contraceptives, phenobarbital, pravastatin, and simvastatin)[5,6]
- Familial hyperalphalipoproteinemia
- Physical exercise
- Pregnancy
- Weight reduction

Apo A1 may be decreased with:

- Chronic renal failure
- Coronary artery disease
- Drugs (e.g., androgens, beta blockers, diuretics, and progestins)
- Familial hypoalphalipoproteinemia
- Smoking
- Uncontrolled diabetes[2]

Clinical References:

1. Fruchart JC, De GC, Delfly B, Castro GR. Apolipoprotein A-I-containing particles and reverse cholesterol transport: evidence for connection between cholesterol efflux and atherosclerosis risk. *Atherosclerosis* 1994 October;110 Suppl:S35-S39.
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replacement therapy on plasma lipids and lipoproteins in postmenopausal women. *Endocr Res* 2000 May;26(2):263-73.

C-Reactive Protein, high sensitivity (hs-CRP)

High sensitivity C-Reactive Protein (hs-CRP) is one of a number of "acute phase reactant" proteins that increases in response to various systemic inflammatory stimuli. Accordingly, any associated medical condition resulting in inflammation, infection, or primary / secondary tissue injury which may cause elevated hs-CRP levels should be considered when interpreting abnormal results in a cardiac risk patient. If hs-CRP levels are >10 mg/L the presence of an acute inflammatory process must be considered. If present, then any elevated hs-CRP secondary to underlying cardiac disease can only be assessed by repeat testing after this acute process has resolved.

Methodology:	Immunoturbidimetry
Performed:	Mon- Fri
Reported:	Within 2 days
Specimen Requirements:	Collect:: One 4 mL serum separator tube. Also acceptable: plasma separator tube, lavender (EDTA), pink (K2EDTA), or green (sodium or lithium heparin). Transport:: 1 mL serum at 2-8°C. (Min: 0.3 mL) Unacceptable Conditions: Hemolyzed specimens. Stability: After separation from cells: Ambient: 2 days; Refrigerated: 2 months; Frozen: 1 year
CPT Code(s):	86141
Reference Interval:	< 3.0 mg/L

Clinical Utility:
Patients with higher hs-CRP concentrations are more likely to develop stroke, myocardial infarction, and severe peripheral vascular disease.

CRP is a nonspecific marker of inflammation and a variety of conditions other than atherosclerosis may cause elevated concentrations. If the first result is greater than 3.0 mg/L, recommend repeating test at least 2 weeks later in a metabolically stable state, free of infection or acute illness. The lower of the two results should be used to determine the patient's risk.

In assessing cardiovascular risk, corresponding to relevant tertiles of adult population distributions, hs-CRP values <1.0 mg/L are considered low risk, values between 1.0 mg/L and 3.0 mg/L are intermediate risk, and values >3.0 mg/L represent a high risk. Due to wide individual test-to-test variability, the protocol to assess individual hs-CRP measurement (and associated risk) recommends obtaining three separate samples, each at least one week apart, and then to calculate the *mean value* as an average[1].

Elevated hs-CRP levels have been associated with a significant increase in risk for cardiovascular events[2-4] This is thought to relate to the fact that atherosclerosis, at least in part, represents an inflammatory response to abnormal lipids in the arterial wall. Even in patients without evidence of CVD, hs-CRP levels in the highest quartile, compared to the lowest quartile, are associated with a 2 to 4 times increased risk of developing subsequent atherosclerotic disease. In combination with elevations in other cardiovascular risk factors (such as elevated LDL-C), this test allows the identification of extremely high risk patients who deserve aggressive treatment[5]. Simultaneous measurements of hs-CRP and a standard lipid profile, including total and HDL cholesterol, predict future vascular risk better than lipid measurements alone. In patients with elevated hs-CRP, aspirin therapy can significantly reduce the risk of future cardiovascular events[6].

Clinical References:

1. de Maat MP, de Bart AC, Hennis BC et al. Interindividual and intraindividual variability in plasma fibrinogen, TPA antigen, PAI activity, and CRP in healthy, young volunteers and patients with angina pectoris. *Arterioscler Thromb Vasc Biol* 1996 September;16(9):1156-62.
2. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000 March 23;342(12):836-43.
3. Lagrand WK, Visser CA, Hermens WT et al. C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? *Circulation* 1999 July 6;100(1):96-102.
4. Ridker PM. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation* 2001 April 3;103(13):1813-8.
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Vitamin D

Most people are familiar with vitamin D's role in preventing rickets in children1 and in helping the body absorb calcium from the diet. Recently, research has shown that vitamin D is important in protecting the body from a wide range of diseases. Disorders linked with vitamin D deficiency include stroke, cardiovascular disease, osteoporosis, osteomalacia, several forms of cancer, some autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, type I diabetes, type II diabetes, depression and even schizophrenia2-12.

Vitamin D is actually a prohormone and not technically a vitamin: a vitamin is defined as a substance that is not made naturally by the body but must be supplied in the diet to maintain life processes. But in fact, we make most of our vitamin D by the action of ultraviolet light (sunlight) on the vitamin D originator that is found in our skin. We only get very small amounts of vitamin D from our diet, although increasingly it is added to foods eaten by children, in an attempt to prevent rickets in the population.

A major cause of deficiency is not getting enough sun. This is very common in northern climates where people don't spend much time outdoors, but even in countries near the equator, women in particular often have much of their skin area covered for cultural reasons, and the use of sunscreen also blocks the formation of vitamin D in the skin.

Vitamin D is metabolized by the liver to a storage form of the vitamin, which circulates in the blood until needed. Enzymes in the kidneys metabolize it further to form the highly active hormone that is involved in essential biochemical processes throughout the body.

Testing for of vitamin D is therefore an important test, especially if you spend much of your time indoors, or live in a colder climate.

Methodology:	Chemiluminescent Immunoassay
Performed:	Mon- Fri
Reported:	Within 2 days
Specimen Requirements:	Collect: : One 4 mL serum separator tube. Also acceptable: lavender (EDTA), pink (K2EDTA), or green (sodium or lithium heparin). Transport:: 1 mL serum at 2-8°C. (Min: 0.3 mL) Unacceptable Conditions: Grossly Hemolyzed or lipemic specimens. Stability: After separation from cells: Ambient: 3 days; Refrigerated: 1 week; Frozen: 6 months
CPT Code(s):	82306
Reference Interval:	Deficiency: Less than 20 ng/mL , Insufficiency: 20-29 ng/mL, Optimum Level: 30-80 ng/mL, Possible Toxicity: Greater than 80 ng/mL

Clinical Utility:
A growing body of scientific data suggests that vitamin D deficiency is associated with increased CV risk.[1] Persons with vitamin D deficiency have been shown to be predisposed to HTN, DM (both types 1 and 2), MetS, LVH, HF, and chronic vascular inflammation.[2] Long-term prospective observational studies, including the US Physicians Health Study and the Framingham Offspring Study, have linked vitamin D deficiency with a 2-fold increased risk of MI [3, 4]. Similarly, in a cohort of 3258 German adults who were followed up for 7.7 years after an elective cardiac catheterization, those in the lowest quartile for baseline serum 25-hydroxyvitamin D had a 2-fold increased risk of CV death compared with those in the highest quartile.[5] A meta-analysis including 18 randomized controlled trials comprising 57,000 participants showed that an intake of vitamin D greater than 500 IU/d was associated with a reduction in all-cause mortality, in part because of a lower incidence of CV deaths.[6] Although a general consensus regarding the optimal levels of serum 25-hydroxyvitamin D does not yet exist, most experts consider a level of less than 20 ng/mL (to convert to nmol/L, multiply by 2.496) deficient and a level of 21 to 29 ng/mL insufficient.[4] Large randomized controlled trials are needed to clarify the relevance of Vitamin D status to CV health. In the interim, monitoring of vitamin D levels in patients with CV disease or CV risk factors (eg, HTN, DM, elevated hsCRP levels) is suggested, and repletion of suboptimal levels is clearly indicated for optimizing musculoskeletal health. Vitamin D deficiency is present in approximately 90% of persons who report symptoms of myalgias while receiving statin therapy.[7]

Treatment of Vitamin D Deficiencies:
In a nonrandomized series from our Preventive Cardiology Clinic at the Mid America Heart Institute, about 80% of such patients can be successfully maintained on a statin when their vitamin D level is normalized via a vitamin D supplement. Repletion of vitamin D should be initiated with 50,000 IU of vitamin D2 or D3 once or twice weekly for a period of 8 to 12 weeks. Vitamin D can be supplemented by sunlight (about 3000 IU of vitamin D3 per 5-10 minutes of midday sun) or 1000 to 5000 IU/d of vitamin D. Among foods, oily fish such as wild salmon have the highest content of vitamin D, ranging from 100 to 1000 IU per 3.5 oz.[4]

Clinical References:
1. Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc*. 2006;81(3):353-373.
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Homocysteine (Hcy)

Homocysteine (Hcy) is a metabolic byproduct of methionine metabolism. Elevated homocysteine is a documented risk factor for cardiovascular events with metabolic interactions and supportive clinical evidence tying homocysteine and CVD. McCully first hypothesized the relationship of homocysteine to atherosclerosis in 1969, which is outlined with metabolic and clinical considerations in his 1998 editorial[2]. A homocysteine level >14 µmol/L is elevated and reflects an increased risk for CAD events. A homocysteine level of 10-14 µmol/L is considered moderately elevated in CAD patients[1]. Elevated levels are associated with a 2-3 fold increased CVD
Risk[1].

Methodology:	Chemiluminescent Immunoassay
Performed:	Mon- Fri
Reported:	Within 2 days
Specimen Requirements:	Collect: : One 5 mL green (sodium or lithium heparin) or serum separator tube. Transport:: 1 mL serum at 2-8°C. (Min: 0.3 mL) Unacceptable Conditions: Sodium citrate. Stability: Ambient: 1 hour; Refrigerated: 1 week; Frozen: 3 months
CPT Code(s):	83090
Reference Interval:	Desirable: Less than 11 µmol/L , At Risk ≥ 14 µmol/L

Clinical Utility:
There is clinical relevance in identifying those patients with elevated homocysteine (and still with compounded CVD risks) as well as the opportunity for appropriate investigative differential diagnosis as to the underlying cause of the abnormality. As one example, it is important to identify these patients and rule out homocysteinemia that is secondary to other causes, such as renal failure or pernicious anemia. Assessing the level of methylmalonic acid (MMA) will help rule out pernicious anemia. Homocysteine may be elevated in association with pernicious anemia. Pernicious anemia may be appropriately treated with B vitamins. Recognizing niacin's potential effect to elevate homocysteine, physicians may want to check the homocysteine level on patients on niacin at baseline and after dose stabilization and consider adjusting therapy in patients with elevated levels.

Treatment of High Homocysteine:
Earlier assumptions about the effectiveness of vitamin B6 and B12 or folate therapy in lowering homocysteine levels have been reversed by more recent publications and meta-data analysis. Loscalzo summarizes today's "state-of-the-art", now based on newer clinical trial data that refutes the original treatment hypothesis[1] (i.e., that since these vitamin supplements lower homocysteine, that treatment should lower CVD events). This editorial discusses some

theoretical reasons why lowering elevated homocysteine with Vitamin B/ folate therapy does not decrease CVD risks.

Clinical References:

1. Malinow MR, Nieto FJ, Szklo M, Chambless LE, Bond G. Carotid artery intimal-medial wall thickening and plasma homocyst(e)ine in asymptomatic adults. The Atherosclerosis Risk in Communities Study. *Circulation* 1993 April;87(4):1107-13.
2. McCully KS. Homocysteine, folate, vitamin B6, and cardiovascular disease. JAMA 1998 February 4;279(5):392-3.

Insulin

Insulin is a protein involved with carbohydrate metabolism. Insulin is elevated postprandially in proportion to the carbohydrate content in a meal. The median value is a fasting serum insulin of 10 μU/ml and ≥12.4 μU/ml is the upper tertile cut-off point. Values in the upper tertile have been associated with increased cardiovascular risk. Values in excess of 15 μU/ml are very elevated.

Methodology:	Chemiluminescent Immunoassay
Performed:	Mon- Fri
Reported:	Within 2 days
Specimen Requirements:	Collect: : One 4 mL serum separator tube. Also acceptable: lavender (EDTA) or pink (K2EDTA) Transport:: 1 mL serum at 2-8°C. (Min: 0.3 mL) Unacceptable Conditions: Heparinized plasma or I.V. fluid. Specimens collected in potassium oxalate/sodium fluoride tubes. Hemolyzed specimens. Stability: After separation from cells: Ambient: 8 hours; Refrigerated: 1 week; Frozen: 1 month
CPT Code(s):	83525
Reference Interval:	To convert to pmol/L, multiply µIU/mL by 6.0.High Fasting insulin is > 12 µlU/mL

Clinical Utility:
Hyperinsulinemia (elevated insulin levels) is associated with the atherogenic lipid profile and the metabolic syndrome. This is a clinically important marker for patients having a family history of diabetes or in those patients having a slight elevation in blood glucose without other symptoms of the disease.

Hyperinsulinemia is associated with a significant increase in the risk for cardiovascular events, but there is an overlap between insulin levels in CAD and control patients. The use of fasting insulin, especially when combined with other risk factors identifies patients at a significantly higher risk for the development of cardiovascular disease. The combination of elevated insulin (>15 μU/ml) and elevated apo B (>119 mg/dL) indicates a very high risk group.

The loss of estrogen at menopause is associated with increased insulin levels. There is an increased cardiovascular (CV) risk when hyperinsulinemia is present incombination with the small, dense LDL trait[1]. There is even a greater risk when Apo B, small, dense LDL and insulin levels are all elevated in combination. In these patients, CV risk is increased 20-fold[2]. Elevated fasting insulin levels have been related to atherosclerosis risk particularly in South Asian Men[3]. In patients with established CAD and elevated insulin levels following a glucose load, there is a significant increased risk for restenosis following angioplasty or coronary stent placement[4,5].

Treatment of High Insulin:
Hyperinsulinemia may be treated with metformin and/or the thiazolodinediones, as clinically indicated[6,7]. Treatment should always include appropriate therapeutic lifestyle adjustment changes such as increased physical activity, loss of excess body fat, and dietary avoidance of simple carbohydrates. Addition of a high-fiber diet should also be considered. For example, a diet high in fiber has been shown to have a beneficial effect on lipids and postprandial insulin in type 2 diabetes patients[8].

Clinical References:

1. Despres JP, Lamarche B, Mauriege P et al. Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med* 1996 April 11;334(15):952-7.
2. Lamarche B, Tchernof A, Mauriege P et al. Fasting insulin and apolipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. *JAMA* 1998 June 24;279(24):1955-61.
3. McKeigue PM, Ferrie JE, Pierpoint T, Marmot MG. Association of early-onset coronary heart disease in South Asian men with glucose intolerance and hyperinsulinemia. *Circulation* 1993 January;87(1):152-61.
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NT- proBNP

The N-terminal fragment of the prohormone B-type natriuretic peptide (NTproBNP) is an endogenously produced neurohormone primarily secreted from the cardiac ventricular myocytes in response to cardiac stress. Clinically, measurements of NT-proBNP are now used in the diagnosis of left ventricular (LV) systolic and diastolic dysfunction (cardiac stretch / stress) and prognostically in a variety of cardiac disease states, including Heart Failure (HF), Acute Coronary Syndrome (ACS), stable coronary artery disease (CAD) and chronic stable angina pectoris. All of these are FDA-approved indications for clinical use. As a highly sensitive marker for cardiac dysfunction, elevated NT-proBNP levels indicate the presence of an underlying cardiac disorder. A normal serum NT-proBNP level virtually excludes cardiac dysfunction. NTproBNP is an independent marker of long-term morbidity and mortality in patients with stable and unstable cardiovascular disease (CVD) at levels well below originally determined cutpoints consistent with frank heart failure. An elevation of NT-proBNP, even in the setting of undiagnosed, subclinical CVD is prognostic of future cardiovascular events.

Importantly, elevated baseline concentrations of NTproBNP are independently related to future adverse events. Thus, even relatively low levels of circulating NT-proBNP may be considered an integrative index of general increased cardiovascular risk for any patient. Determinations of baseline NT-proBNP levels are an important adjunct in a clinician's evaluation for a secondary prevention, at-risk CVD population in a disease management setting.

Methodology:	Chemiluminescent Immunoassay
Performed:	Mon- Fri
Reported:	Within 2 days
Specimen Requirements:	Collect: : One 4 mL serum separator tube. Transport:: 1 mL serum at 2-8°C. (Min: 0.3 mL) Unacceptable Conditions: Hemolyzed specimens. Stability: After separation from cells: Ambient: 4 hours; Refrigerated: 4 hours; Frozen: 4 months
CPT Code(s):	83880
Reference Interval:	To convert to pmol/L, multiply µIU/mL by 6.0.High Fasting insulin is > 12 µIU/mL

Clinical Utility in Symptomatic Cardiac Disease:
Baseline levels of NT-proBNP are independently related to future cardiovascular events in patients with stable angina pectoris[1,2] NT-proBNP is a marker of long-term mortality in patients with stable CAD; it provides prognostic information above and beyond conventional CV risk factors and the degree of LV dysfunction[3,4] . A normal serum NT-proBNP level virtually

excludes cardiac dysfunction. As a sensitive marker of cardiac function, elevated NT-proBNP levels resulting from cardiomyocyte stress indicate an underlying cardiac disorder. As discussed above, NT-proBNP is effectively used as an aid in the diagnosis of left ventricular systolic and diastolic dysfunction (cardiac stress) and has prognostic value in a variety of symptomatic cardiac disease states, including ACS and HF. Elevated plasma concentrations of NT-proBNP have been shown to be an excellent independent predictor of cardiac events in a variety of clinical situations, as well as a predictor of cardiac and non-cardiac mortality. NT-proBNP levels are independently associated with increased mortality even after adjustment for clinical phenotypes and echocardiographic abnormalities. NT-proBNP rises in response to cardiac ischemia. Even without a change in diastolic filling pressures during a cardiac catheterization and percutaneous coronary intervention (PCI), there is a rapid rise in NT-proBNP levels following balloon inflation (temporary coronary occlusion).[5] At the same time, it does not require a documented ischemic event to find abnormal levels in asymptomatic patients, which becomes highly significant for diagnosis and even prognosis. When NT-proBNP levels are elevated before stress testing in asymptomatic patients with CAD, it predicts those subjects who subsequently have a positive test result.[6,7] This finding is independent of underlying LV systolic function and a history of prior myocardial infarction.[6]

Clinical Utility in Asymptomatic Cardiac Disease:
There is also now a supportable diagnostic and prognostic role for NT-proBNP in patients with chronic stable CAD, substantially contributing to the assessment of increased risk for mortality and CVD events in this patient population, even in association with subclinical disease.[8-12] Measuring NT-proBNP levels can now stand as a valuable clinical tool to identify patients with early stage, often pre-clinical, cardiac dysfunction.[13] As such, NTproBNP can be used as an early marker of long-term mortality in patients with asymptomatic, stable CAD and provide prognostic information above and beyond that correlated to conventional cardiovascular risk factors or a given degree of left ventricular dysfunction.[14]

NT-proBNP is an independent and an additive biomarker for clinical diagnosis and prognosis in a variety of cardiac disease states, including heart failure, acute coronary syndrome and chronic stable coronary artery disease. NT-proBNP predictably identifies cardiac dysfunction in asymptomatic patients in a non-ER setting to target earlier therapeutic intervention. The FDA-approved NT-proBNP cutpoints of 125/450 pg/mL are effective to investigate the early diagnosis of left ventricular dysfunction and for ruling-out heart failure in the physician's office. There is abundant literature supporting the role for evaluating natriuretic peptides as prognostic and diagnostic cardiovascular biomarkers. Both BNP and NT-proBNP have proven efficacy for diagnosing patients with ventricular dysfunction and for obtaining prognostic information in those with heart failure, CHD and ACS. From a laboratory perspective, NT-proBNP is a more stable analyte in vivo and in vitro than BNP.

Studies are emerging to show a broader clinical utility for NT-proBNP in less symptomatic patients, i.e., those with earlier stage disease. Existing data anticipates the role of NT-proBNP as a discriminating prognostic and diagnostic marker in earlier-stage cardiac patients with subclinical disease. By identifying unsuspected cardiac (cardiomyocyte) structural damage earlier in the disease process, clinicians can better identify unsuspecting patients predicted at high risk for future cardiac events and mortality. These patients are in need of an early, more

comprehensive, diagnostic cardiac workup and may be candidates for early initiation of pharmacologic treatment with drugs targeting cardiac dysfunction. Extensive data supports the resultant lowering of plasma NTproBNP levels when standard approaches, including drug therapy, are used to treat LV dysfunction. It is important to remember that a falling NT-proBNP level after an episode of treated LV failure significantly reduces later cardiac morbidity and mortality.

After adjusting for traditional risk factors, NT-proBNP remains an independent, strong predictor of cardiovascular disease and death. Even after accounting for left ventricular systolic dysfunction, the NT-proBNP level is a robust predictor of morbidity and mortality. Stable patients with underlying CAD can have elevated NT-proBNP levels and these levels relate to the extent of CAD. NT-proBNP values can also predict the extent of CAD in patients with preserved left ventricular function. In addition, NT-proBNP measured at the time of angiography is a predictor of long-term risk of death in patients with stable ischemic heart disease. After adjustment for LV structural and functional parameters and evidence of symptomatic coronary ischemia, there is an independent association between coronary atherosclerotic burden, as measured by the CAC score, and plasma NT-proBNP levels. These findings support the hypothesis that coronary atherosclerosis may directly lead to activation of the cardiac neurohormonal system as a subclinical driver of eventual left ventricular dysfunction. Clinical and research data anticipate a much broader role for NT-proBNP in diverse cardiac and even non-cardiac risk groups, used in a manner beyond many other validated prognostic markers—as a standard metric to better identify those at risk, but earlier in the stage of the disease process. The natural clinical extension of these data will look to support NT-proBNP as a metric to follow therapeutic interventions. This will allow physicians to initiate an early and more aggressive, targeted patient treatment program to improve eventual cardiovascular prognosis. Incorporating NT-proBNP testing into standard practice has the potential to positively impact more than 500,000 patients annually in the U.S. In addition to improving clinical outcomes over time, there is also data that this management will be associated with overall economic benefit with reduced healthcare costs.[30]

NT-proBNP also displays a very high negative predictive value allowing low values to rule out cardiac disease. Multiple studies have shown that incorporating NTproBNP testing in a patient evaluation is far superior to clinical judgment alone. This important advantage allows clinicians to refine their diagnostic success to better identify a patient at risk for appropriate therapy and intervention. Earlier diagnosis will translate into better outcomes and the opportunity of slowing, or even regressing, a cardiovascular disease process that is affecting cardiac function.

Elevated NT-proBNP—Therapeutic Considerations

Multiple medications used to treat cardiac dysfunctional disorders are known to affect circulating levels of cardiac natriuretic peptides. Drug effects may have a role in altering cardiac natriuretic peptide secretion and/or affect plasma clearance of the peptide. Clinicians must consider these effects when interpreting and/or treating elevated plasma levels of NT-proBNP. A recent comprehensive review of drug effects on circulating levels of natriuretic peptides has been written by Troughton *et al.*[28] The effect of medications on cardiomyocyte secretion rates depends on a complex sum interaction of multiple signals that change

ventricular stretch, in particular transmural chamber pressure. This dominant induction signal is additively influenced by stimulus from other sources including the renin-angiotensin system, the sympathetic nervous system (stimulatory alpha-1 agonists and inhibitory beta-agonists), thyroid hormone, vasopressin, prostaglandins, hypoxia/ischemia and cytokines.69 For example, angiotensin-converting enzyme (ACE) inhibitors reduce left ventricular stretch in the failing heart while also lowering angiotensin II and sympathetic response feedback. The interaction of these signals combine to decrease cardiomyocyte natriuretic peptide secretion. A more complex interaction results from the use of betablockers in the failing heart.[29] In the short term, beta-blockers increase ventricular stretch by inhibiting beta-agonism, increasing natriuretic peptide levels. This level is further increased as beta-blockade reduces plasma clearance. But in the long term, chronic beta-blockade leads to beneficial ventricular remodeling decreasing chamber stretch which decreases peptide secretion. In summary, natriuretic peptides levels are suppressed by oral statins, diuretics, renin-angiotensin system blockers, vasodilators and intravenous dopamine-like agents and amiodarone. Beta-blocker effects are variable, increasing natriuretic peptides in hypertensive subjects and often showing a biphasic effect in heart failure subjects with minimal change or increasing levels seen in the short term and lowering of levels in the longer term. This reflects the temporal remodeling process of a failing ventricle with continued beta blockade.

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