Update on Selected Markers Used in Risk Assessment for Vascular Disease

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INTRODUCTION

The current Focus section includes three articles associated with updates on various cardiac markers. The initial article, entitled, "Update on Selected Markers Used in Risk Assessment for Vascular Disease" by Eileen Carreiro-Lewandowski, provides information on selected markers used in preventative medicine for identifying and establishing treatment plans for patients at increased risk of vascular disease. Alan Wu, from Hartford Hospital, CT and a well-known authority in cardiac marker utilization, authors the second article providing essential information on troponin assay issues. The last article, written by Debra Faubion, provides information regarding BNP testing.

ABBREVIATIONS: ATPIII = Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III); CHD = coronary heart disease; CVD = cardiovascular vascular disease; ET-1 = Endothelin 1; HDL = high density lipoprotein cholesterol; HRT = hormone replacement therapy; Hs-CRP = high-sensitivity C-reactive protein; LDL = low density lipoprotein cholesterol; NO = nitric oxide; PAF = potent platelet-activating factor; TG = triglycerides; TLC = therapeutic lifestyle changes.

INDEX TERMS: coronary heart disease.

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Focus Continuing Education Credit: see pages 57 to 60 for learning objectives, test questions, and application form.

LEARNING OBJECTIVES

1. Define endothelium dysfunction.
2. Describe the interrelationship of endothelium dysfunction and CVD.
3. Discuss the LDL, total cholesterol, HDL, and triglyceride levels recommended in the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) guidelines.
4. Describe the major risk factors, exclusive of LDL levels and existing CHD, that may modify LDL therapeutic goals.
5. List the conditions defining the metabolic syndrome.
6. List the guidelines for lipoprotein testing.
7. Discuss the apparent relationship of omega-3 fatty acid levels and sudden death.
8. Compare and contrast the terms "risk factor" and "risk marker".
9. List the key factors that influence the predictive value and clinical utility of blood test markers.
10. Discuss the use of hs-CRP as an indicator of CHD.
11. List several inflammatory markers, excluding hs-CRP, that may be used to indicate possible risk of CVD.
12. Discuss some of the recent findings in non-inflammatory CVD testing.

Vascular disease, including that leading to heart disease, stroke, or other thrombotic related disorders, continues to plague the public at alarming rates. The estimated annual costs of cardiovascular disease in the USA and Canada are $298 billion and $20 billion, respectively.[1] More recently, atherosclerosis development and progression have been strongly linked to inflammatory processes. The term "car-

diovascular vascular disease" (CVD) often represents a myriad of symptoms affecting multiple vascular territories including major arteries associated with the heart and brain, and most likely other organs such as the kidneys, plus the peripheral circulation.[2] It is estimated that transient ischemic attacks, whether cardiac, cerebrovascular, renal, or vascular, have not been included in many major studies, and the real incidence of vascular disease is much higher than reported. A constellation of lipid and nonlipid risk factors, including those referred to as the 'metabolic syndrome', clearly links development of diabetes mellitus and CVD. One of the risk factors, obesity, has reached epidemic proportions in the U.S., further contributing to the incidence of CVD. Laboratory markers are increasingly important in identifying those individuals at greatest risk of CVD and determining treatment modalities for these patients.

ENDOTHELIAL DYSFUNCTION AND CVD

The endothelium is a single-cell lining covering the internal surface of blood vessels, cardiac valves, and body cavities. Vascular homeostasis is maintained by a balance of vasoactive substances (Table 1) released by the endothelium. This balance maintains vasomotion, smooth muscle proliferation, thrombosis, inflammation, coagulation, fibrinolysis, and oxidation. Endothelium dysfunction occurs when one or more of these functions is disrupted, and the careful balance between these vasoactive substances is disturbed. Generally, the imbalance is associated with either diminished availability or production of nitric oxide (NO), which mediates the vasodilatory action of acetylcholine and maintains vascular smooth muscle tone. Nitric oxide opposes the actions of vasoconstricting peptides such as angiotension-II, norepinephrine, serotonin, and endothelin-1(ET-1), and inhibits platelet and leukocyte activation. An imbalance of the effectiveness of any of these endothelium derived substances can also cause endothelial dysfunction.

Table 1. Selected endothelium vasoactive related substances

ACTION	SUBSTANCE
Antithrombic	Nitric oxide (NO), plasminogen activator, protein C, tissue factor inhibitor, von Willebrand factor
Prothrombic	Endothelin 1 (ET-1), **oxidant radicals**, plasminogen-activator, inhibitor-1, thromboxane A2, fibrinogen, tissue factor
Vasoconstrictors	ET-1, **angiotensin-II**, thromboxane B, **oxidant radicals**, prostaglandin H_2
Vasodilators	NO, prostacyclin, bradykinen, adrenomedullin, natriuretic peptides
Permeability	Receptor for advanced glycosylation end-products
Antiproliferative	NO, prostacyclin, transforming growth factor-b, heparin
Proliferative	ET-1, **angiotensin II**, **oxidant radicals**, platelet-derived growth, factor, insulin-like growth factor, interleukins

BOLD PRINT: Substances listed in more than one area
Taken from: Verma S, Anderson T. Fundamentals of endothelial function for the clinical cardiologist. Circ 2002;105;546-51.

Endothelial dysfunction has been implicated in the pathogenesis and clinical course of all known CVD and is associated with future risk of adverse CVD events.[3] Endothelium injury occurs in response to the same risk factors connected to CVD development. Conditions associated with impaired endothelial function include atherosclerosis, hypercholesterolemia, hypertension, increased homocysteine levels, vasculitis, pre-eclampsia, metabolic syndrome, diabetes, active and passive cigarette smoking, ischemia-reperfusion, post menopause, infections, depression, lack of physical activity, obesity, renal failure, the aging process, and congestive heart failure. Adverse changes in lipoproteins associated with CVD, including small-dense, low-density lipoproteins, are also linked to impaired endothelial function.

Many of the laboratory and clinical tests under close scrutiny and development attempt to measure the degree of endothelium dysfunction and by association, the process and/or stage of atherosclerosis. This is because endot-

helial imbalance participates in lesion formation by promotion of the vasculature to vasoconstriction, leukocyte adherence, platelet activation, mitogenesis, enhanced LDL oxidation, thrombosis, impaired coagulation, vascular inflammation, increased cell permeability, and cytokine elaboration. It also participates in the process of lesion formation, plaque instability, and rupture. As the biochemistry of bodily stress is elucidated, other factors may be implicated in establishing an environment for the destructive effects of endothelium dysfunction.

LIPID UPDATE

While the exact involvement of lipid levels in predicting all individuals at increased risk for CVD remains controversial, the National Cholesterol Education Program's Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III/ATP III) Executive Summary provides guidelines focused on lowering the LDL cholesterol as the primary treatment target.[4] Key features include using a full lipoprotein profile consisting of total cholesterol, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), and serum triglyceride levels. It also defines associated major risk factors independent of the LDL that may modify the LDL levels for individuals at risk for developing coronary heart disease (CHD). Moreover, it acknowledges the influence of other factors, termed life-style and emerging factors, that may influence the individual's risk of CHD independent of the impact they may have on LDL levels.

The new guidelines for cholesterol, LDL, HDL, and triglycerides are provided in Table 2. The major risk factors, exclusive of LDL, that may modify LDL therapeutic goals are listed in Table 3. An individual's risk score is determined based on the Framingham Point Scores and then placed into one of three 10-year risk categories with varying LDL goals (Table 4). It should be noted that diabetes mellitus infers the same risk equivalent as does a CHD event. Other CHD risk equivalents include other forms of atherosclerotic disease, and a combination of multiple risk factors that confer a risk of greater than 20% of CHD within a 10-year period. A second category consists of individuals in whom there is between a 10%

Table 2. ATP III lipid guidelines* (mg/dL)

	Optimal	Near/above optimal	Borderline high	High	Very high
LDL	<100	100-129	130-159	160-189	≥190
Total Cholesterol	<200 (desirable)		200-239	≥240	
HDL	<40† (low)			≥60	
Triglycerides	<150 (normal)		150-199	200-499	≥500

* Based on 9- to 12-hour fast
† <50 for women suspected of metabolic syndrome

Table 3. Major non-LDL CHD risk factors

Cigarette smoking

Hypertension (BP ≥140/90 or on antihypertensive medication

Low HDL (<40 mg/dL)

Family history of premature CHD (CHD in male first degree relative <55 years; female first degree relative <65 years)

Diabetes

Age (male ≥45 years; female ≥55 years)

Table 4. LDL goals and risk category

Risk category	LDL goal (mg/dL)
CHD or CHD equivalent risk (Framingham 10-year risk >20%)	<100
2+ risk factors (10-year risk <20%)	<130
0-1 risk factors (10-year risk <10%)	<160

to 20% 10-year risk for CHD having multiple (two or more) risk factors. The risk scores are determined based on the Framingham Point Scores. Individuals fall into one of three risk categories with varying LDL goals (Table 4).

Unique to the latest version of the ATP III, are guidelines for a constellation of risk factors that contribute to clinical identification of the metabolic syndrome (Table 5). This is a major concern since it is estimated that between 22% to 24% of U.S. adults have the metabolic syndrome.[5] While there was little difference between Caucasian males and females, ethnicity appears to have an impact on the incidence of the metabolic syndrome. Prevalence was highest among Mexican Americans, followed by African-American women. The metabolic syndrome appears closely linked to insulin resistance, a fundamental defect in type 2 diabetes, and altered biochemistry of lipid metabolism associated with increased triglyceride levels, excess body fat, and abdominal obesity.[6] The ATP III reports that the risk factors associated with the metabolic syndrome collectively increase risk of CHD, regardless of the associated LDL levels.

The ATP III recommends that lipoprotein testing should occur in all adults starting at the age of 20, and repeated at five year intervals. It recommends that an initial panel include all four tests (total cholesterol, LDL, HDL, and triglycerides) and testing be performed on fasting samples. In the event a sample is procured from a non-fasting individual, only the HDL and total cholesterol can be considered. If the total cholesterol values are ≥200 mg/dL or HDL is <40 mg/dL, it is recommended that a full, fasting, lipoprotein panel then be performed.

Table 5. Risk factors associated with metabolic syndrome*

Factor	Definition
Abdominal obesity/ insulin resistance	Waist circumference
Male	>40 in
Female	>35 in
Triglycerides	≥150 mg/dL
HDL cholesterol	
Male	<40 mg/dL
Female	<50 mg/dL
Blood pressure	≥130/≥85 mmHg
Fasting glucose	≥110 mg/dL

* ATP III guideline defines metabolic syndrome as any three of the above

The report also proposes a multifaceted approach to reducing the risk of CHD, depending on the individual's assessed category of risk. These include lipid-lowering substances, such as prescription medications, use of plant sterols, and diets with increased soluble fiber, along with 'therapeutic lifestyle changes' (TLC) such as weight reduction, increased physical activity, and a TLC diet consisting of 35% of daily calories from fat, mostly from unsaturated sources.

Lower levels of long chain omega-3 polyunsaturated fatty acids found in fish are associated with an increased risk of sudden death among survivors of myocardial infarction.[7] While findings remain inconclusive, sufficient data lead to a statement by the American Heart Association recommending the inclusion of fish in a healthy diet.[8] Concerns as to the purity of the fatty acids and the risk of adverse effects related to high levels of mercury and other accumulated products in some fish may place additional testing burden in the domain of the clinical laboratory.

NONLIPID MARKERS

Inflammatory markers

Atherosclerosis as an inflammatory response has been previously well established.[9,10] Major factors associated with promoting atherosclerosis are also associated with increased risk of inflammation and endothelium dysfunction. These include adverse level and type of lipoproteins, cigarette smoking, hypertension, insulin resistance associated with an increased glucose/diabetes, and the metabolic syndrome as previously mentioned. Recent interest has focused on the potential use of a number of inflammatory related analytes in an attempt to identify either risk factors, defined as the measurement of substances that may lead to atherosclerosis, or risk markers, described as analytes produced as a consequence of the disease process itself (Table 6). While the difference in the terminology may seem minor, the classification of a substance as either a risk factor or marker may cause significant changes in patient treatment and care.

Regardless of its use in risk assessment, whether for detection of atherosclerosis, related processes, or any other so called blood markers, e.g., so called 'tumor markers', the predic-

tive value and clinical utility of any laboratory test is influenced by a number of key factors. These include: 1) analytical specificity and sensitivity; 2) assay associated total error; 3) ability to standardize assay methods and associated calibration material; 4) diagnostic efficiency for identifiable select populations; 5) independence from other risk factors or markers; 6) the relationship between the analyte and its clinical endpoint; and 7) acceptable assay cost and availability. One test that has recently received considerable study is high-sensitivity C-reactive protein (hs-CRP).

CRP is an early acute phase reactant. It increases in response to active infections, systemic inflammatory processes, or trauma. Lower levels of CRP are evident in chronic inflammatory situations, such as that associated with atherosclerotic processes or its development. Traditional assays lacked the sensitivity to monitor changes in chronic inflammation, and as a result, hs-CRP assays were developed. Acute inflammatory changes are associated with increased levels of hs-CRP (≤10 mg/L) and the underlying etiology should be investigated. It has been suggested that a repeat of the hs-CRP, as an independent indicator of CVD risk, should only occur after the acute inflammatory episode has resolved, allowing for a minimum of a two week interval between testing.[11,12]

Table 6. Selected inflammatory related substances identified for potential marker use

Proinflammatory risk factors
- Oxidized LDL
- Cytokines
  - Interleukins
  - Tumor necrosis-factor

Cellular adhesion molecules (CAMs)
- Intercellular adhesion molecule-1
- Selectins (P-, L-, and E-)
- b2 integrins

Inflammatory response related proteins
- Serum amyloid A (SAA)
- C-reactive protein
- Fibrinogen
- Platelet-activating factor acetylhydrolase

Cellular response
- White blood cell count

While hs-CRP may serve as a useful predictor of increased CHD, widespread screening of the adult population as a public health measure is not recommended at this time.[3,13] Guidelines for hs-CRP utilization published by the CDC/American Heart Association indicate that results should be uniformly expressed in units of mg/L.[14] Using standardized assays, cutpoints of low risk (<1.0 mg/L), average risk (1.0 to 3.0 mg/L), and high risk (>3.0 mg/L) correspond to tertiles based on population distributions (40,000 individuals) of hs-CRP when reviewed by this working group. Based on these findings, coupled with other recent scientific studies, this same group recommended that hs-CRP serve as the representative atherosclerotic inflammatory marker, but only in certain circumstances. Risk assignment should only occur in those individuals free from other inflammatory conditions, in those patients with an intermediate 10-year CHD risk (10% to 20% Framingham Risk Score/ATP III guidelines), in the absence of other major risk factors including known CHD, or at the discretion of the physician, particularly in those patients having baseline risk but for whom further guidance is needed when establishing risk assessment or a treatment plan. The assay may be useful as an independent marker in patients with stable coronary disease, stroke, peripheral artery disease, acute coronary syndromes, or for assessing the likelihood of recurrent events, such as restenosis or death.[15]

In addition to the presence of inflammatory disease, hs-CRP has several limitations. Little data exists for racial and ethnic populations. The use of estrogen in hormone replacement therapy (HRT) is associated with increased hs-CRP levels and LDL levels, however, the link of HRT and increased hs-CRP to increase CHD risk or incidence has not been established.[16] Increased body weight, body mass index, elevated blood pressure, cigarette smoking, low HDL/high triglycerides, and the metabolic syndrome are associated with increased hs-CRP, while weight loss, moderate alcohol consumption, and increased physical activity, particularly endurance exercise, reduces hs-CRP levels. The mechanisms are poorly defined, but these associations bring into question the role of insulin-resistance and its relationship to hs-CRP and its contribution to CHD.

Promising data exists for increased hs-CRP as a significant risk factor when used in combination with the HDL/total cholesterol in women with values within suggested guidelines.[17] In a more recent study reported by Ridker, hs-CRP and LDL cholesterol (direct-measurement assay) were measured in all of the participants in the Women's Health Study (27,939 women over 45 years of age).[18] Their data suggests that hs-

CRP may be superior to LDL in predicting the risk of first cardiovascular events, including stroke, nonfatal myocardial infarction, coronary revascularization procedures, and death from cardiovascular causes in this population even after other risk factors, including HRT, were accounted for in the results. Seventy-seven percent of first cardiovascular events in the study occurred in those with LDL levels below 160 mg/dL, and 46% occurred in those with levels below 130 mg/dL.

Changes in other inflammatory markers (Table 6), particularly the white-cell count, and fibrinogen levels, are also associated with risk of coronary events, but their predictive ability is limited when present with other CVD risks and availability of testing. Lipoprotein-associated phospholipase A2 (Lp-PLAC2) detection, like hs-CRP, may hold promise as an independent marker for indicating increased risk in persons who are otherwise considered as low risk. Lp-PLAC2, also known as plasma platelet-activating acetylhydrolase (PAF-AH)/(EC 3.1.1.47), belongs to a family of enzymes which degrade and inactivate the biologically potent platelet-activating factor (PAF). PAF is produced by, but also responds to, many sources including mast cells, platelets, basophils, neutrophils, monocytes/macrophages, and endothelium. In addition to atherosclerosis, inflammation, and ischemia-reperfusion, PAF has been linked to asthma, diabetes, enterocolitis and irritable bowel syndrome, arthritis, corneal ulcers, infant death, and reproductive dysfunction. Lp-PLAC2/PAF-AH is released and attempts to mitigate the harmful effects of PAF and may be protective. It also functions to degrade oxidized phospholipids in circulating plasma lipoproteins further serving as a protective substance.[19] This role contrasts with reports of its pro-inflammatory actions, possibly as a result of the products of substrate degradation, namely oxidized free fatty acids and lysophosphatydyl choline, both of which are considered high inflammatory mediators.[20,21] Conflicting data concerning Lp-PLAC2/PAF-AH as a significant independent risk factor and/or a promoter of atherosclerosis has yet to be resolved.[22,23] Further research might shed light on this proposed marker. Regardless of the result, its expression and role in inflammation may be altered by pre-existing risk factors (abnormalities in LDL, insulin-resistance, etc.), genetic changes, or similar to the dynamics seen in endothelium dysfunction, an indication of an imbalance of substances within the vasculature.

NON-INFLAMMATORY MARKERS

Elevated homocysteine levels continue to be of interest as an indicator of increased CVD risk, and development of dementia and Alzheimer's Disease.[24] A causal relationship has not been established. It is particularly intriguing because studies show that levels can be decreased, along with decreased rate of coronary restenosis, using a combination of folic acid, vitamins B_{12}, and B_6 even after other risk factors are considered.[25,26] Homocysteine facilitates the oxidation of LDL cholesterol, and the possible promotion of endothelium dysfunction, however, the exact mechanisms are unknown. The causes of high serum homocysteine concentrations include genetic defects in homocysteine metabolism, alterations in vitamin B_{12} metabolism, and dietary folate deficiency. Genetic variations related to transcobalamin, the substance responsible for vitamin B_{12} transportation into the cell, may be more susceptible to vitamin B_{12} treatment for lowering increased homocysteine levels.[27]

The differences between males and females in assessing CVD risk remains a mystery. Studies regarding HRT as a means of decreased incidence of CVD have yielded conflicting results. Reduced iron stores associated with menstruation has been given some consideration. The idea that iron overload may contribute to increased CVD due to the formation of oxidized free radicals, has been refuted using angiography and measurements of ferritin, yet more recent animal studies indicate that a "moderate iron loading markedly accelerates thrombus formation after arterial injury, increases vascular oxidative stress, and impairs vasoreactivity".[28,29] Much of the risk assessment data to date is based on predominantly male populations. Questions arise regarding the effectiveness of the current risk assessment markers in pre- and post-menopausal female populations. Do real differences between the sexes exist or does research need to focus on a different set of indicators for this population?

SUMMARY

CVD remains the greatest health risk in the U.S.. Assessment of laboratory data in establishing risk and treatment modalities has come to the forefront in patient primary care. Guidelines published in the ATP III document by the NCEP have incorporated lower limits of lipids and included a number of risk factors and conditions, such as the metabolic syndrome associated with insulin-resistance, as a means for earlier detection and intervention in CVD. Endothelial dysfunction and the associated inflammatory process, including soluble plasma markers, have lead to the addition of hs-CRP as an adjunct to other laboratory indicators of CVD. The precise mechanisms and interrelationships between these factors and atherosclerosis have yielded some confusing data, along with investigations of a number of associated substances and conditions. An emerging theme is the body's response

to injury and stress; a lack of metabolic balance. While currently outside the domain of routine laboratory testing, future CVD risk assessment may include the metabolic byproducts generated by chronic external pressures, including genetic predisposition or alterations associated with socioeconomic factors. Further studies are needed to better understand the significance each plays in assessing the individual's development and CVD risk.

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