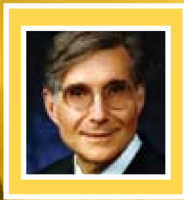
CMEdiscovery.com™ *presents...*

Inflammatory Aspects and Detection of Vulnerable Plaque

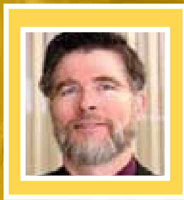
Clinical Impact of Assessment

CHAIR

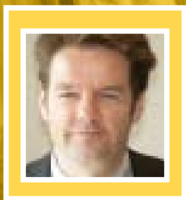


PETER LIBBY, MD
Mallinckrodt Professor of Medicine
Harvard Medical School
Chief, Cardiovascular Medicine
Brigham and Women's Hospital

FACULTY



JOHN COOKE, MD, PhD
Professor of Medicine
Stanford University
School of Medicine



ANTONIUS F. W. VAN DER STEEN, PhD
Head Biomedical Engineering
Thorax Centre
ErasmusMC, Rotterdam

This program is supported by an unrestricted educational grant from Bristol-Myers Squibb Medical Imaging

Jointly sponsored by ArcMesa Educators and Scinexa, LLC

Inflammatory Aspects and Detection *of*

CHAIR

PETER LIBBY, MD

Mallinckrodt Professor of Medicine
Harvard Medical School
Chief, Cardiovascular Medicine
Brigham and Women's Hospital

FACULTY

JOHN COOKE, MD, PhD

Professor of Medicine
Stanford University
School of Medicine

ANTONIUS F. W. VAN DER STEEN, PhD

Head Biomedical Engineering
Thorax Centre
ErasmusMC, Rotterdam

TABLE OF CONTENTS

Release Date: December 15, 2006
Expiration Date: October 23, 2008

MEDICAL WRITER

WENDY GLOFFKE, PhD

This program is supported by an unrestricted educational grant from Bristol-Myers Squibb Medical Imaging.

Jointly sponsored by
ArcMesa EDUCATORS scinexa™

The views and opinions expressed in this activity are those of the faculty. They do not reflect the views of Bristol-Myers Squibb Medical Imaging, any other manufacturer of pharmaceuticals, ArcMesa Educators, or Scinexa, LLC. The recommendations made in this program are based upon a combination of randomized clinical trials, current guidelines, and the clinical practice experience of the participating panelists. Any medications, diagnostic procedures or treatments discussed by the panelists should not be utilized without evaluation of their patient's conditions. Participants are urged to consult the full prescribing information on any drug mentioned in this activity for recommended dosage, indications, contraindications, warnings, precautions, and adverse effects before prescribing any medication.

Vulnerable Plaque *Clinical Impact of Assessment*

PROGRAM DESCRIPTION

Rupture-prone (i.e. "vulnerable") plaques are a primary cause of cause of acute coronary syndromes and myocardial infarction. Though the features of rupture-prone atherosclerotic plaques have been described by pathologists, the identification of high-risk plaque in vivo remains a challenge. While coronary angiography is the gold standard for diagnosis of a plaque, alternative detection methods that rely upon physical, optical, and mechanical parameters may help to direct treatment decisions and improve clinical outcomes.

TARGET AUDIENCE

This educational initiative is intended for physicians and other health care professionals who specialize in the field of interventional vascular therapy.

EDUCATIONAL OBJECTIVES

Following this educational activity participants will:

- Discuss what constitutes a vulnerable or high-risk plaque
- Review the role of inflammation and composition of vulnerable plaque
- Discuss the rationale for both invasive and non-invasive visualization techniques to assess vulnerable plaque
- Describe how plaque assessment potentially impacts prognosis and treatment

ACCREDITATION

This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of ArcMesa Educators and Scinexa, LLC. ArcMesa Educators is accredited by the ACCME to provide continuing medical education for physicians.

ArcMesa Educators designates this educational activity for a maximum of *2 AMA PRA Category 1 credits*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Disclosure Policy

It is the policy of ArcMesa Educators to ensure balance, independence, objectivity, and scientific rigor in all its educational activities. All faculty/authors are expected to disclose any relevant financial relationships they may have with commercial interests in relation to this activity. These relationships, along with the educational content of this program, have been reviewed and any potential conflicts of interest have been resolved to the satisfaction of ArcMesa Educators.

Faculty Disclosure Declarations

Peter Libby, MD– Consultant, Speakers' Bureau: AstraZeneca, Bristol-Myers Squibb, Merck & Co., Inc, Novartis, Pfizer, and Sanofi-Aventis; Consultant: Interleukin Genetics, Schering Plough, and GlaxoSmithKline

John Cooke, MD, PhD– Speaker's Bureau: Bristol-Myer Squibb; Grant Support: Genentech; Stock: Athenager; Research Support: Genzyme and Ciphergen

Antonius F. W. van der Steen, PhD– Research Support: Volcano Corp, Boston Scientific, LightLab, BMS Imaging

Wendy Gloffke, PhD– Nothing to disclose

Staff Disclosure Declarations

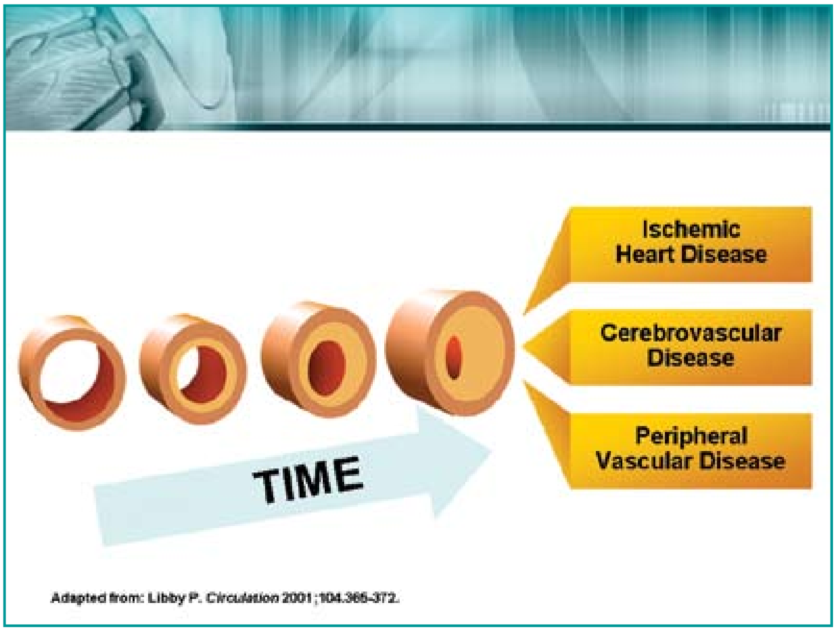
The employees of Scinexa Medical Education Company and ArcMesa Educators have no financial relationships to disclose.

Inflammatory Aspects and Detection *of*

Introduction

Atherosclerosis has traditionally been described as developing via a sequence of events in the artery wall along a time continuum; cholesterol-laden debris accumulates on the artery wall, progressively occluding the lumen of the artery. In this model, stenotic plaques that compromise the coronary arteries by more than 60%-70% place a patient at higher risk for an acute coronary syndrome. Retrospective data including morphologic evidence, pathology studies, serial angiograms, and emerging human data are changing this view of atherosclerosis. Mounting evidence suggests that plaque progression and clinical outcome are not always correlated.

Figure 1. Traditional view of the sequence of events that lead to coronary artery disease.



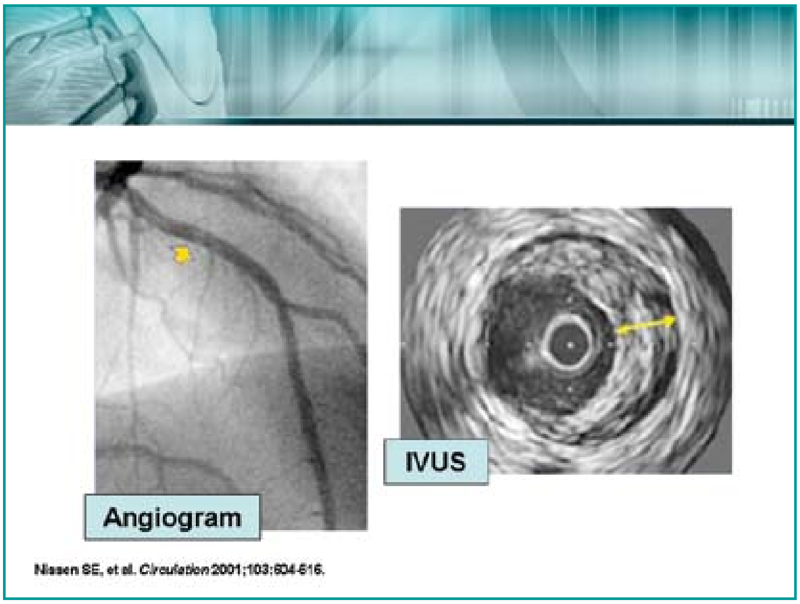
Vulnerable Plaques: Pathogenesis and Composition

Peter Libby, MD

In the traditional view of atherogenesis, the development of stenosis causes the transition from chronic stable or asymptomatic atherosclerosis, which can endure for decades in humans, to acute disease, with the potential for thrombotic complications and sudden death (Figure 1). Serial angiographic studies, however, have demonstrated that the coronary arteries with severe stenosis (i.e., > 70%) do not cause the majority of acute myocardial infarctions (MIs). Instead, many acute MIs occurred in arteries that did not previously contain significant stenosis.[1,2] Studies over the past decade have shown that most plaques that underlie a fatal or nonfatal MI are less than 70% stenosed angiographically.[3,4] Maseri and colleagues studied 60 consecutive patients assessed with quantitative coronary arteriography (QCA) during their MI after thrombolysis. The researchers were surprised to find that once the offending thrombus had been lysed, the residual stenosis was noncritical (<60%) in almost half the cases.[5] The Maseri results were corroborated by serial angiographic studies that evaluated prior angiograms in patients who had an MI. Only about 15% of the regions that caused myocardial infarction had a flow-limiting stenosis greater than 70% on an antecedent angiogram.[6] Two thirds of myocardial infarctions occurred at sites that, on the previously-recorded angiogram, showed a hemodynamically "insignificant" lesion or "minor irregularity" that would be classified as noncritical.[6] These and other clinical data led researchers to conclude that compensatory enlargement resulted in asymptomatic disease despite atheroma bulk, due to outward remodeling of the affected artery. A lack of ischemia concealed the disease from the patient, and the absence of defects on angiograms hid it from the physician. Using intravascular ultrasound (IVUS), however, researchers have shown that atherosclerosis commonly lurks behind the shadow of a normal angiogram (Figure 2).

Vulnerable Plaque *Clinical Impact of Assessment*

Figure 2. Intravascular ultrasound shows atheromas that are not detected by angiography.



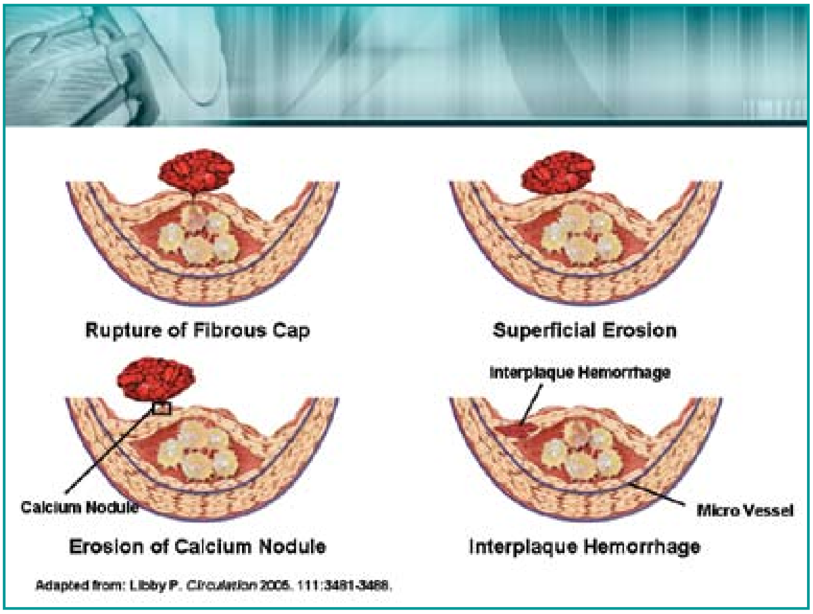
A description of plaques that are vulnerable to rupture, or (so called "vulnerable plaques", or VPs), has emerged from anatomical and pathological research and examination of plaques that have caused acute fatal MI (Figure 4). Compared to stable plaques, VPs generally have a larger lipid core (>40% of total lesion area), a thinner fibrous cap (65-150 micrometers), and many inflammatory cells. Finally, there is a notable paucity, rather than an abundance, of smooth muscle cells.

Pathogenesis of Vulnerable Plaque

The molecular and cellular biology that underlies the weakening of the fibrous cap is complex, and appears to involve numerous inflammatory and enzymatic processes (Figure 5). Many matrix-degrading proteases, including metalloproteinases and elastolytic cathepsins, participate in atherogenesis, and proteolysis may predispose plaque to disruption and thrombosis.

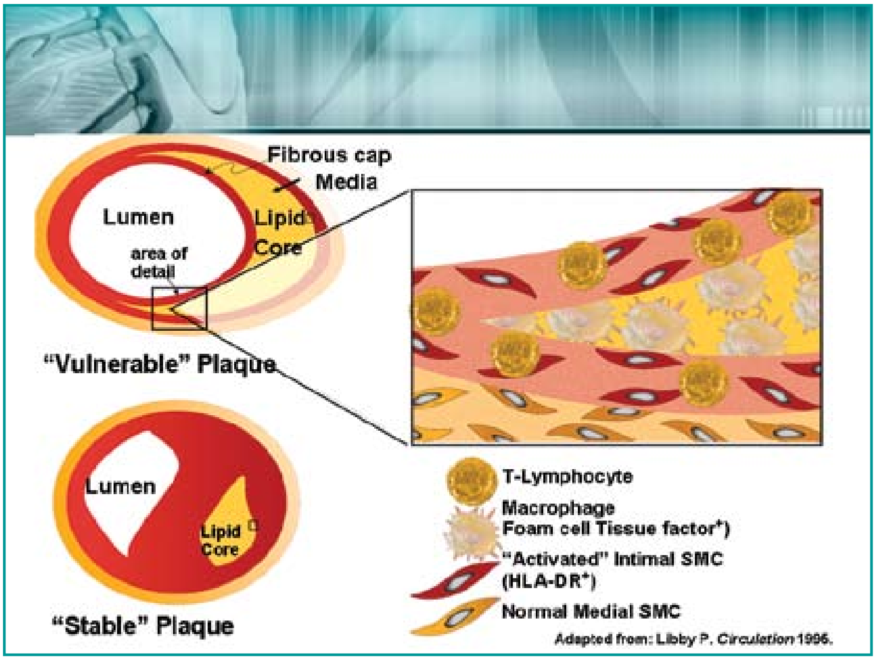
Mechanical disruption of an atherosclerotic plaque, not stenosis, is the most common cause of an acute thrombotic occlusion that leads to death in patients. While four mechanisms of plaque disruption can occur, the majority (66%-75%) of acute fatal MIs are due to rupture of the fibrous cap (Figure 3). Another important mechanism, superficial erosion, causes about one fifth (at most, one quarter) of acute fatal myocardial infarctions. Erosion of calcium nodules and intraplaque hemorrhage, while interesting, are much less common as a cause of fatal myocardial infarction.

Figure 3. Four mechanisms of plaque rupture.



Inflammatory Aspects and Detection *of*

Figure 4. The differences between "vulnerable" and stable plaques.



Interstitial collagen fibrils, derived from smooth muscle cells, crucially determine the integrity of the plaque's fibrous cap. As smooth muscle cells synthesize these collagen fibrils, we can understand why plaques that are vulnerable to rupture seem to have few smooth muscle cells. Ultimately, inflammation in the intima (depicted by the T-lymphocyte in gold or the macrophage foam cell in beige in Figure 5) produces molecular signals that are sent to the smooth muscle cell. These signals inhibit new collagen production in smooth muscle cells and render them unable to repair and maintain the collagen that runs straight to the plaque's fibrous cap.

The stability of the fibrous cap is sensitive to the activity of metalloproteinases, including the gelatinase, MMP-9, that break down the extracellular matrix. Biochemical studies *in situ*, studies in atherosclerotic mice, and clinical correlates in humans suggest that MMP-9 is an important molecule in the process of plaque rupture and may be a biomarker for outcomes in patients with coronary artery disease (CAD). Biochemical studies have shown that the active forms of MMP-9 are present in extracts of human atherosclerotic plaques, but are not present in noninvolved vessels. Local overexpression of an active form of MMP-9, induced with a viral vector, produced plaque rupture in atherosclerotic mice.[7] Among patients with coronary artery disease, those who had the highest plasma levels of MMP-9 had the highest incidence of death.[8] Elastolytic cathepsins, a non-metalloproteinase family of enzymes that are overexpressed in atherosclerosis, are also involved in plaque proteolysis.[9]

Normal arteries do not express cathepsins, but all major cell types in human atheroma express cathepsins K and S.[9]

At the D.W. Reynolds Center at Harvard, Professor Ralph Weissleder from the Center for Molecular Imaging at the Massachusetts General Hospital has developed selective probes for MMP-9 and cathepsin K that can be used *in vivo* to identify inflammed plaques using near-infrared fluorescence (NIRF). The MMP-9 probe produces very low background fluorescence until it is activated by gelatinases, at which point it fluoresces. In our collaborative studies in atherosclerotic mice, atheromata produced a fluorescent signal whose intensity correlated with histological evidence of MMP-9 (Figure 6). Atherosclerotic mice also showed that cathepsin K immunoreactivity correlated with a near-infrared signal and with macrophages, but not smooth muscle cells (unpublished data).

In human carotid endarterectomy specimens, NIRF probes show cathepsin K and macrophages in athersclerotic lesions. This suggests that NIRF may be a practical technique for invasive diagnosis in the carotid and deeper arteries; our Reynolds Center team is developing catheters that will be able to detect the NIRF signal.

As Times/CNN's Dr. Sanjay Gupta wrote" "Vulnerable plaque soft, hidden, and deadly is changing the way that doctors think about cardiac disease". Ongoing research is seeking to develop tools that could allow *in vivo* monitoring of inflammation and enzymatic activities to further understand and detect the disease, to evaluate therapies, and to improve patient outcomes.

Vulnerable Plaque *Clinical Impact of Assessment*

Vulnerable Blood: Biomarkers Associated with Plaque Rupture

John Cooke, MD, PhD

Blood with an increased level or activity of circulating thrombogenic factors is referred to as “vulnerable”.[10] Traditional biomarkers for cardiovascular risk include low-density lipoprotein (LDL) cholesterol and glucose. However, 50% of heart attacks and strokes occur in individuals that have normal LDL cholesterol by current guidelines, and 20% of major adverse cardiovascular events occur in patients with no accepted risk factors.[11] In light of changing atherosclerosis models, vulnerable blood may be better described as blood that has an increased level or activity of plasma determinants of plaque progression/rupture.

Proposed biomarkers of plaque progression and rupture fall into eight general categories: inflammatory markers, markers for plaque erosion and thrombosis, lipid-associated markers, markers of endothelial dysfunction, myocardial injury or dysfunction markers, oxidative stress, metabolic markers, and markers of neovascularization (Figure 7). In any individual patient, however, it is not yet clear how these biomarkers relate to the quantitative risk of a major adverse cardiovascular event.

The processes involved in plaque rupture include endothelial dysfunction activation, lipid accumulation, oxidative stress and inflammation, matrix remodeling, neovascularization and thrombosis. Ideally, a biomarker should reflect the underlying biological processes associated with plaque burden or disease progression. A useful biomarker also needs to be reliable and accurate, sensitive and specific for the disease process, independently predictive of major adverse cardiovascular events (i.e., independent of already accepted markers or risk factors), and cost-effective.

Characteristics of a Clinically Useful Biomarker

- Reflects plaque burden or progression
- Reliable and accurate
- Sensitive, specific
- Independently predictive of MACE
- Cost-effective

Markers of Inflammation

Markers of inflammation include C-reactive protein (CRP), soluble CD40 ligand (sCD40L), soluble vascular cell adhesion molecules (sVCAM), and tumor necrosis factor (TNF). The prototypic marker for inflammation is C-reactive peptide, which has been shown to be an independent predictor of adverse cardiovascular events in patients with risk factors and in patients with disease. Statin-induced reduction of CRP is associated with less progression in adverse cardiovascular events that is independent of the lipid-associated changes.[12] The Centers for Disease Control and American Heart Association guidelines recommend that CRP be used to stratify those patients who are at intermediate risk by the Framingham criteria.[13-17]

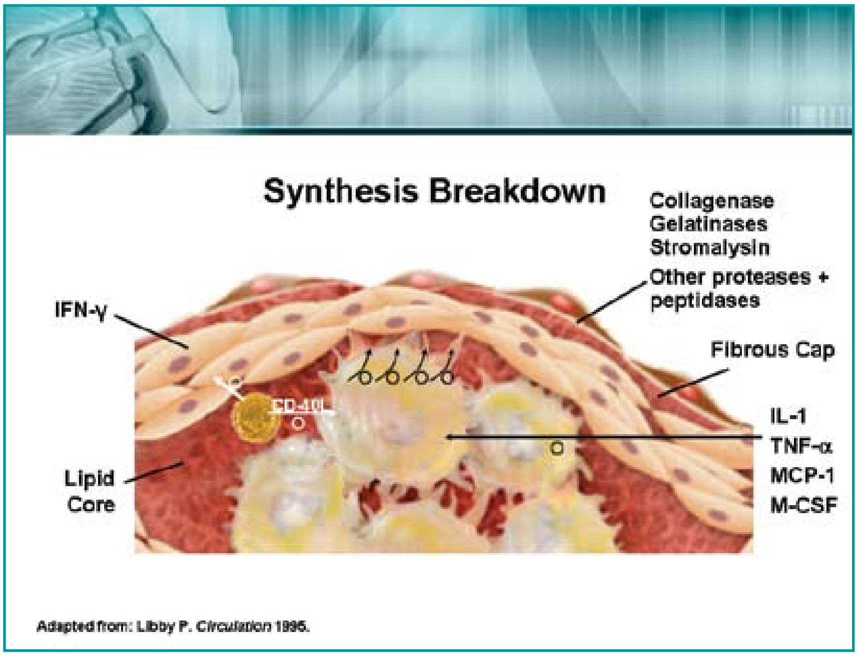
Soluble CD40 ligand (sCD40L) is believed to be released from activated platelets and is a potential biomarker for smalldisruptions, endothelial denudation, or fissures. Accurate, standardized assays need to be developed in order to evaluate sCD40L, sVCAM, and TNF as biomarkers.

Lipid Markers

Lipid markers, in addition to LDL cholesterol and high-density lipoprotein (HDL) cholesterol, include oxidized LDL cholesterol, small dense LDL cholesterol, lipoprotein (a), and lipoprotein-associated phospholipase A_2.

Oxidation of LDL cholesterol typically occurs in the diseased vessel wall and plays a role in foam cell formation. There are different forms of oxidized

Figure 5. Inflammation in the pathogenesis of acute coronary syndromes.



Inflammatory Aspects and Detection *of*

LDL cholesterol, depending upon which component, the apoprotein or lipid, is oxidized. Each form is detected by a different antibody. Oxidized LDL cholesterol is increased in the setting of plaque rupture, whether due to acute coronary syndrome or percutaneous intervention. Elevations in oxidized LDL cholesterol can be detected almost immediately after intervention, which suggests it is being released by the vessel wall. Increased oxidized LDL cholesterol is associated with increases in carotid intimal medial thickness and impaired flow-mediated vasodilation. It is predictive of CAD, progression, and major acute coronary events (MACE).[18-21]

Small dense LDL cholesterol, also known as the "Pattern B" by gel electrophoresis, is a more easily oxidized form of LDL cholesterol. It is very common with insulin resistance and hypertriglyceridemia, which raises the question of whether it truly provides more information than just a triglyceride level.

Lipoprotein (a) is a unique lipoprotein, similar to LDL cholesterol except for an additional apoprotein (a) that is homologous to plasminogen. The homologous units are involved in fibrinogen binding and may, therefore, be prothrombotic by interfering with the action of plasmin on fibrin. Levels over 30 mg/dL are predictive of coronary artery disease and major adverse cardiovascular events. Lipoprotein (a) levels are not responsive to a lipid-lowering diets or statins, but are responsive to niacin. Patients under 50 years of age with premature peripheral arterial disease and premature coronary artery disease tend to have a high prevalence of lipoprotein (a).[22-26]

Lipoprotein-associated phospholipase A_2 (LP-PLA_2) is somewhat controversial, but recent epidemiological studies suggest it is linked with major adverse cardiovascular events. Lipoprotein-associated phospholipase A_2 is associated with lipid, particularly LDL cholesterol, and cleaves it to produce oxidized fatty acids and lysolecithin, which can also be inflammatory mediators. The question of whether it is a risk marker that is truly independent from LDL cholesterol remains to be answered.

Figure 6. Detection of gelatinase activity *in vivo* in the aorta of atherosclerotic mice.

Metabolic Markers

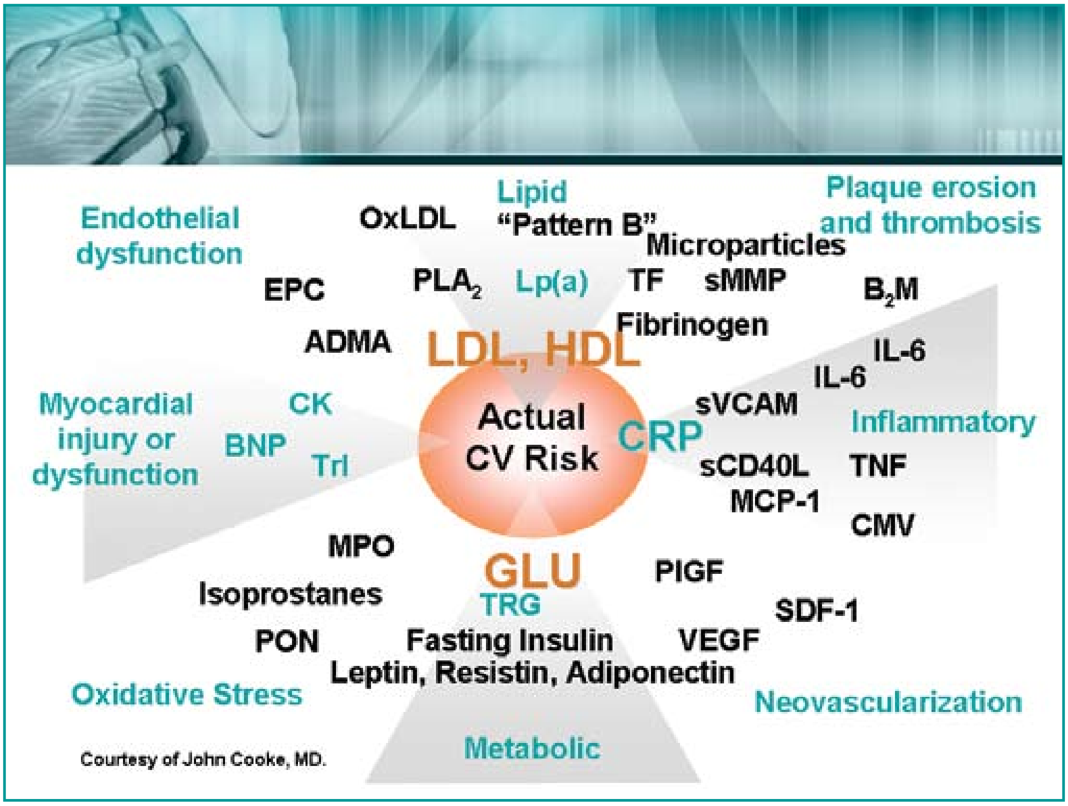
Insulin and glucose are classic metabolic biomarkers of insulin resistance. Recent research has focused on adipokines that may be involved in atherogenesis, including leptin and resistin, and inflammatory cytokines released by adipose tissue (e.g., TNF) or in response to their release (CRP).[27] Adiponectin an adipose-derived cytokine that appears to be vasoprotective, and may be a prognostic marker for a good cardiovascular outcome.

Endothelial Dysfunction

Numerous studies have confirmed that endothelial vasodilator dysfunction is independently predictive of cardiovascular events.[28] Acetylcholine releases nitric oxide, prostacyclin, and other vasodilators from the endothelium; vasodilation is the normal response to acetylcholine. Results from studies of acetylcholine-induced vasoreactivity in patients undergoing catheterization showed that patients with endothelial vasodilator dysfunction exhibited vascoconstriction, rather than vasodilation, in response to acetylcholine. These patients also had a worse prognosis than patients who responded normally.[29]

Potential markers of endothelial dysfunction include nitric oxide, asymmetric dimethyl-arginine (ADMA), nitrogen oxides (NOx), sVCAM, von Willebrand factor, and endothelial progenitor cells. Nitric oxide, which is a vasodilator, is also a vasoprotective molecule that inhibits smooth muscle cell proliferation, leukocyte adhesion, and platelet adherence and aggregation. It is derived from the metabolism of arginine by endothelium nitric oxide synthase (eNO-synthase) to nitric oxide.

Figure 7. Potential biomarkers of plaque progression and rupture.



In the circulatory system, ADMA, an arginine analog, competes with arginine and inhibits the production of nitric oxide. Studies show that ADMA is elevated in individuals with cardiovascular risk factors. Accumulating evidence suggests that circulating ADMA may itself predispose toward cardiovascular events, and therefore act as a marker of risk.[30-32]

Nitrogen oxides can be measured directly in the plasma or urine, but the test results can be obscured by the presence of nitrates and nitrites in food and water. Soluble adhesion molecules and von Willebrand factor are known to be increased with endothelial injury or dysfunction.

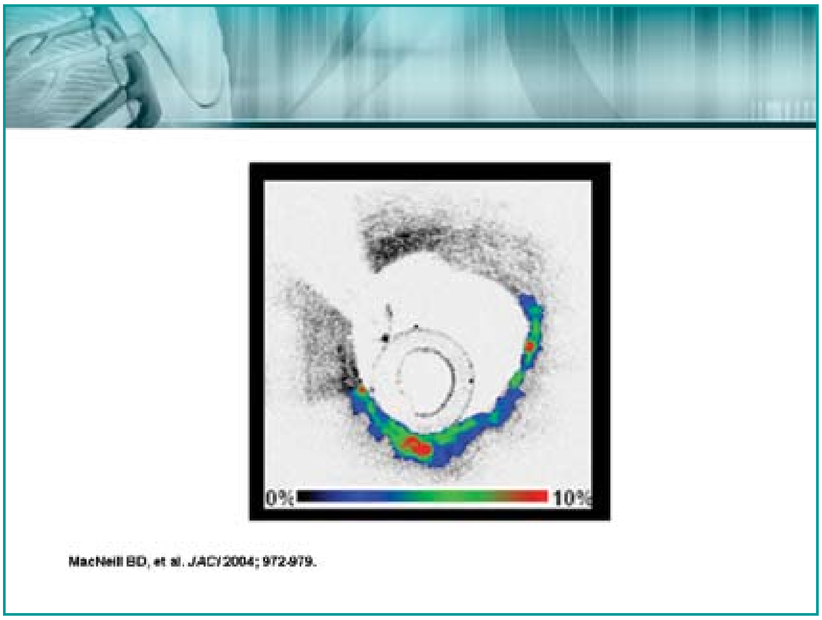
Endothelial progenitor cells (EPCs) are bone marrow-derived stem cells for the endothelium and for the vascular smooth muscle that can resurface injured endothelium or participate in angiogenesis.[33] Recent work indicates that the number of endothelial progenitor cells is inversely correlated to ADMA and MACE, and is directly correlated to endothelial vasodilator function. Fewer circulating EPCs mean less replicative capacity and increased risk of a major adverse cardiovascular event; more EPCs mean better endothelial vasodilator function.[34] Since it is difficult to measure EPCs directly, researchers are examining potential biomarkers of circulating EPCs, including sKit ligand and stromal derived factor, which are increased and participate in mobilizing endothelial progenitor cells from the bone marrow.

Oxidative Stress

Oxidative stress plays a very important role in atherogenesis. Evidence shows that the activation of vascular oxidative enzymes (e.g., NADPH oxygenase, myeloperoxidase) in the vessel wall that is initiated by angiotensin-2 (AII), glucose, advanced glycosylating end products (AGE), or infectious agents.[35] Activation of these oxidative enzymes leads to lipid oxidation, foam cell formation, expression of vascular adhesion molecules and chemokines, and atherogenesis. Isoprostanes, myeloperoxidase, and oxidized LDL cholesterol are all markers of oxidative stress.

Inflammatory Aspects and Detection *of*

Figure 8. Optical coherence tomography shows macrophage density in a plaque.



Nicotine is an angiogenic agent and also plays a role in plaque progression. In the apoE-deficient, hypercholesterolemic mouse model, nicotine increases plaque growth with increased neovascularization.[46,47] The nicotine receptor, a ligand-gated calcium channel on the vascular endothelial cells, plays a role in angiogenesis when it is stimulated. Nicotine can "hijack" the endogenous cholinergic pathway, causing pathological angiogenesis.[46, 47]

Many molecules have been identified as potential biomarkers of plaque progression and rupture. While the traditional markers such as CRP are known to be independent markers of cardiovascular risk, the predictive value of many of the others has not yet been proven. Ultimately, the best outcomes may be achieved using a panel of markers that will capture all of the different processes involved in plaque progression and plaque rupture, and that will enable clinicians to quantify an individual patient's true cardiovascular risk.

Isoprostanes are the gold standard for oxidative stress.[36] They are the end products of the oxidation of arachidonic acid, which is derived from lipid of cell membranes and lipoproteins. Isoprostanes are elevated with inflammation and percutaneous procedures in acute coronary syndromes and are cardiovascular (CV) risk factors. The fact that they are difficult to measure and provide no prognostic information has limited their clinical use, and they are primarily a research tool.

In contrast, myeloperoxidase (MPO), which appears to be very involved in atherogenic processes, is relatively easy to measure. Myeloperoxidase generates free radicals (hypochlorous anion (HOCl), tyrosyl radicals) that can accelerate foam cell formation. It is heavily expressed at sites of plaque rupture. Serum MPO levels are not only predictive of endothelial dysfunction, but also a strong and independent predictor of major adverse cardiovascular events in patients with CAD or acute coronary syndrome (ACS).[37-40]

Plaque Erosion and Thrombosis

Tissue factor (TF), a thrombogenic protein made by macrophages, plays a very important, perhaps dominant, role in thrombosis subsequent to plaque rupture. Tissue factor is highly concentrated in the lipid core, of plaque. An emerging hypothesis is that TF released from a diseased vessel wall becomes part of a circulating pool of TF. Plasma levels of TF are increased 2 hours after smoking and are also elevated with CV risk factors.[10,41]

Neovascularization

Emerging research suggests that plaque neovascularization plays a role in plaque growth and progression.[42] As such, angiogenic cytokines including placental growth factor (PLGF) and stromal-derived factor 1 (SDF-1) may be potential biomarkers for the process. Placental growth factor has been shown to be a predictor of major adverse cardiovascular events.[43-45]

Techniques for Assessing Vulnerable Plaques and Clinical Applications

Antonius F.W. van der Steen, PhD

The identification of vulnerable plaques is challenging from an engineering standpoint. Any practical modality must differentiate between calcified, fibrous, and fatty tissue; identify the thin fibrous cap surrounding the plaque and provide an estimate of its thickness; and identify inflammation. It is imperative that a technique should have good parametric, spatial (axial, lateral, elevational), and temporal resolution and provide accurate data in a timely manner. Many devices are able to adequately discriminate calcified tissue from other tissue, but are not sensitive enough to distinguish between fibrous tissue and a lipid core in a plaque. Those devices that are more sensitive tend to be affected by motion, noise, and electrical noise. Design of vulnerable plaque detection devices should take all of these aspects into account.

Vulnerable Plaque *Clinical Impact of Assessment*

Non-Invasive Techniques for Assessing Vulnerable Plaque

Multislice computed tomography (MSCT) is an X-ray–based technology with good temporal resolution that has been improved in the next generation of CT scanners. Volumetric CT scanners will have a temporal resolution under 50 milliseconds, a scan time of just one heartbeat, and an isotropic resolution (all three dimensions) of 0.2 millimeter. While this level of sensitivity can detect a fibrous cap, it will not be enough to identify an inflamed thin-cap fibroatheroma (TCFA), and some data can be lost in signal processing.

Magnetic resonance imaging (MRI) techniques and images continue to be improved. Better signal-to-noise and contrast-to-noise ratios allow visualization of smaller vessels and branches. Though in-plane resolutions of 0.6 mm x 0.6 mm are adequate for differentiating some plaque features, out-of-plane resolution (slice thickness) is 1.5 mm. This is not sensitive enough to identify thin caps on the order of 100 microns.[48]

Invasive Techniques for Assessing Vulnerable Plaque

While noninvasive technologies are getting better at detecting vulnerable plaque, at this point in time, invasive technologies provide the best methods for the detection of vulnerable plaque in coronary arteries. Technologies included in this group are optical coherence tomography (OCT), intravascular MRI, near-infrared (NIR) spectroscopy, virtual histology, palpography, and intravascular ultrasound (IVUS). Most of these techniques are used primarily in research settings and clinical studies, and are not yet available in clinical practice.

Optical coherence tomography is a light-based technique that has excellent spatial resolution and can identify thin fibrous caps (Figure 8) and detect macrophages. Since OCT cannot scan through blood, the vessel of interest must first be cleared of blood by flushing or with a balloon.

Furthermore, there are developments to show the location and density of macrophages in a plaque based on speckle analysis in OCT images (Figure 9).[49] Researchers are studying macrophage density in patients with ST-elevated MI, acute coronary syndrome, and stable angina. Using OCT, they have shown that macrophage density is higher in acute clinical syndromes and lower in the stable group. In addition to the requirement that a vessel be cleared of blood, another limitation of OCT is that it cannot image the entire vessel at one time.

Near-infrared spectroscopy (NIR) is another light-based technique that employs an IVUS-like rapid-exchange coronary catheter that rotates the light beam. Spectra acquisition is rapid, about 5 milliseconds. Absorbance is plotted against wavelength to produce a spectrum that correlates to attenuation through the tissue. A well-validated method, NIR is frequently used to assess the chemical composition of the vessel wall. When used in autopsy specimens, TCFA sensitivity and specificity was greater than 85%. Unlike OCT, NIR is able to scan through blood. The availability of laser, fiber-optic, and chemometric technologies make intracoronary use feasible.

Though MRI has a limited resolution, as previously discussed, intravascular MRI is a newer catheter-based real-time technique that does not require external magnets. Intravascular MRI has a high sensitivity and specificity for differentiating fibrous tissue and lipid rich necrotic cores. The technique requires time to position the catheter with a balloon inside the vessel wall. Also, because it is a somewhat "noisy" technique, data assessment requires relatively more time.

Virtual histology using intravascular ultrasound (VH IVUS) relies upon a radiofrequency analysis of the intravascular ultrasound spectra to identify plaque components including fibrous tissue, fibrous fatty tissue, the necrotic core, and dense calcium. This technique allows much more complete lesion assessment, including plaque composition, on top of the traditional IVUS plaque descriptors, minimal luminal diameter (MLD), proximal and distal vessel diameter, and plaque dimensions themselves.

A new technique called palpography uses ultrasound to "palpate" a vessel from the inside. The images are correlated to levels of deformation (strain) using Young's Modulus (E), which provides a measure of relative hardness that differentiates tissue components.[50,51] Atheroma, for example, is lipid-rich and soft, having an E of 4 kPa; fibrous intimal tissue is more than 100 times harder (E=483 kPa), and calcified regions are 1000 times harder (E=4000 kPa) than atheroma.[52] A high degree of deformation may be associated with a thin-cap fibrous atheroma. Palpography has been shown to be very sensitive and specific, detecting TCFA 90% of the time.[53] In addition to the thin cap, it captures information about other hallmarks of VP including macrophages and smooth muscle cells.[50] Results from *in vitro* studies showed that higher levels of macrophages, fewer smooth muscle cells, and thinner caps all produced higher strain levels. *In vivo* studies revealed that the number of high strain spots in a vessel was directly related to the patient's condition and to CRP levels.[54]

In a study that utilized integrated technologies (multislice CT, grayscale IVUS, virtual histology, palpography, biomarkers) to monitor stable, unstable, and ST-elevated MI patients (N=52), palpography was the only technology that was able to show a treatment effect.[55] The ST-elevated MI group showed a 50% reduction in the Schaar, Mastik, van der Steen (SMS) index, which is a measure for the instability of a vessel based on palpographic measurements, at 6 months compared to baseline.[55] In contrast, compared to baseline, neither the stable nor the unstable group showed significant changes in SMS index at 6 months. These results probably reflect the fact that 11 of the 12 patients in the ST-elevated MI group were naïve to medication at baseline, while patients in the other groups were already being treated with medications (primarily statins) at baseline.[55]

Contrast agents and harmonic IVUS have successfully shown microvasculature in atherosclerotic rabbit aortas.[56,57] This technique transmits ultrasound at 20 MHz and receives at 40 MHz, which enhances the contrast bubble signal while suppressing the tissue signal. Another application being evaluated is the use of harmonic IVUS to look directly at contrast agent bubbles in order to obtain molecular images of their locations.[57, 58]

Though they show promise for clinical applications, with the exception of IVUS technology, these modalities are at present not part of routine clinical practice. They are, however, providing insight into the processes and players associated with vulnerable plaque and are suggesting potential new clinical applications that could improve diagnostic and treatment options in many therapeutic areas.

Potential Applications of Invasive Techniques in the Detection of Vulnerable Plaque

- Optical Coherence Tomography (OCT)
  - Identifies thin fibrous cap
  - Potential for macrophage detection
- Intravascular magnetic resonance imaging (MRI) and near-infrared (NIR) spectroscopy:
  - Identify lipid pools
- Virtual Histology
  - Potential for identification of the relevant components
- Palpography
  - Measures strength of the fibrous cap
  - Measures condition of the vessel wall
- Intravascular Ultrasound (IVUS) contrast
  - Potential for vasa vasorum detection
  - Potential for molecular imaging

Summary

The concept of "vulnerable plaque" is changing the way that clinicians view atherosclerosis. Researchers are teasing out the processes that lead to the development of VP. Retrospective studies helped to identify features associated with VP and researchers are learning how to identify VP using experimental technologies. Potential biomarkers that offer predictive, clinically useful information about VP and disease progression are being evaluated. The role of imaging in VP therapy is still a matter of debate; however, there's no doubt that a combination of imaging and biomarkers will be important in clinical trials and drug development. As imaging technologies make their way into clinical practice, they will probably be used in combination with low resolution parametric methods to improve diagnostic accuracy. Ultimately, these research activities will determine what treatments are indicated, when they should be initiated, and how to better quantify an individual patient's risk for acute cardiovascular events.

References

1. Little WC, Constantinescu M, Applegate RJ, et al. Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease? *Circulation*. 1988;78(5 Pt 1):1157-1166.
2. Ambrose JA, Tannenbaum MA, Alexopoulos D, et al. Angiographic progression of coronary artery disease and the development of myocardial infarction. *J Am Coll Cardiol*. 1988;12(1):56-62.
3. Kullo IJ, Edwards WD, Schwartz RS. Vulnerable plaque: pathobiology and clinical implications. *Ann Intern Med*. 1998;129(12):1050-1060.
4. Virmani R, Burke AP, Farb A, et al. Pathology of the vulnerable plaque. *J Am Coll Cardiol*. 2006;47(8 Suppl):C13-C18.
5. Hackett D, Davies G, Maseri A. Pre-existing coronary stenoses in patients with first myocardial infarction are not necessarily severe. *Eur Heart J*. 1988;9(12):1317-1323.
6. Reiner JS, Lundergan CF, Fung A, et al. Evolution of early TIMI 2 flow after thrombolysis for acute myocardial infarction. *Circulation*. 1996;94(10):2441-2446.
7. Gough PJ, Gomez IG, Wille PT, et al. Macrophage expression of active MMP-9 induces acute plaque disruption in apoE-deficient mice. *J Clin Invest*. 2006;116(1):59-69.
8. Lubos E, Schnabel R, Rupprecht HJ, et al. Prognostic value of tissue inhibitor of metalloproteinase-1 for cardiovascular death among patients with cardiovascular disease: results from the AtheroGene study. *Eur Heart Journal*. 2006;27:150-156.
9. Sukhova GK, Shi GP, Simon DI, et al. Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. *J Clin Invest.* 1998;102(3):576-583.
10. Fuster V, Moreno PR, Fayad ZA, et al. Atherothrombosis and high-risk plaque: part I: evolving concepts*. J Am Coll Cardiol*. 2005;46(6):937-54.
11. Tsimikas S, Willerson JT, Ridker PM. C-reactive protein and other emerging blood biomarkers to optimize risk stratification of vulnerable patients. *J Am Coll Cardiol*. 02006 Apr 18;47(8 Suppl):C19-C31.
12. Ridker PM, Cushman M, Stampfer MJ, et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336(14):973-979.
13. Morrow DA, Rifai N, Antman EM, et al. C-reactive protein is a potent predictor of mortality independently of and in combination with troponin T in acute coronary syndromes: a TIMI IIA substudy. Thrombolysis in Myocardial Infarction. *J Am Coll Cardiol*. 1998;31(7):1460-1465.
14. Ridker PM, Rifai N, Rose L, et al. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med*. 2002;347(20):1557-1565.
15. Sabatine MS, Morrow DA, de Lemos JA, et al. Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes: simultaneous assessment of troponin I, C-reactive protein, and B-type natriuretic peptide. *Circulation*. 2002;105(15):1760-1763.
16. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003;107(3):499-511.
17. Ridker PM, Cannon CP, Morrow D, et al. C-reactive protein levels and outcomes after statin therapy. *N Engl J Med*. 2005;352(1):20-28.
18. Holvoet P, Harris TB, Tracy RP, et al. Association of high coronary heart disease risk status with circulating oxidized LDL in the well-functioning elderly: findings from the Health, Aging, and Body Composition study. *Arterioscler Thromb Vasc Biol*. 2003;23(8):1444-1448.
19. Tsimikas S, Bergmark C, Beyer RW, et al. Temporal increases in plasma markers of oxidized low-density lipoprotein strongly reflect the presence of acute coronary syndromes. *J Am Coll Cardiol*. 2003;41(3):360-370.
20. Tsimikas S, Lau HK, Han KR, et al. Percutaneous coronary intervention results in acute increases in oxidized phospholipids and lipoprotein(a): short-term and long-term immunologic responses to oxidized low-density lipoprotein. *Circulation*. 2004;109(25):3164-3170.
21. Liu J, Thewke DP, Su YR, et al. Reduced macrophage apoptosis is associated with accelerated atherosclerosis in low-density lipoprotein receptor-null mice. *Arterioscler Thromb Vasc Biol*. 2005;25(1):174-179.
22. Ridker PM, Hennekens CH, Stampfer MJ. A prospective study of lipoprotein(a) and the risk of myocardial infarction. *JAMA.* 1993;270(18):2195-2199.
23. Schaefer EJ, Lamon-Fava S, Jenner JL, et al. Lipoprotein(a) levels and risk of coronary heart disease in men. The lipid Research Clinics Coronary Primary Prevention Trial. *JAMA*. 1994;271(13):999-1003.
24. Maher VM, Brown BG, Marcovina SM, et al. Effects of lowering elevated LDL cholesterol on the cardiovascular risk of lipoprotein(a). *JAMA*. 1995;274(22):1771-1774.
25. Danesh J, Collins R, Peto R. Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. *Circulation*. 2000;102(10):1082-1085.
26. Ariyo AA, Thach C, Tracy R. Lp(a) lipoprotein, vascular disease, and mortality in the elderly. *N Engl J Med*. 2003;349(22):2108-2115.
27. Cooke JP, Oka RK. Does Leptin cause vascular disease? *Circulation*. 2002;106(15):1904-1905.

28. Cooke JP. Flow, NO, and atherogenesis. *Proc Natl Acad Sci USA*. 2003;100(3):768-770.

29. Zeiher AM. Endothelial vasodilator dysfunction: pathogenetic link to myocardial ischemia or epiphenomenon. *Lancet*. 1996;348:s10–s12.

30. Meredith IT, Yeung AC, Weidinger FF, et al. Role of impaired endothelium-dependent vasodilation in ischemic manifestations of coronary artery disease. *Circulation*. 1993;87(suppl V): V56–V66.

31. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation*. 2000;101(16):1899-1906.

32. Cooke JP. Asymmetrical dimethylarginine: the Uber marker? *Circulation*. 2004;109(15):1813-1818.

33. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997;275(5302):964-967.

34. Hill JM, Zalos G, Halcox JP, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med*. 2003;348(7):593-600.

35. Guzik TJ, Harrison DG. Vascular NADPH oxidases as drug targets for novel antioxidant strategies. *Drug Discov Today.* 2006;11(11-12):524-533.

36. Pratico D, Rokach J, Lawson J, et al. F2-isoprostanes as indices of lipid peroxidation in inflammatory diseases. *Chem Phys Lipids*. 2004;128(1-2):165-171.

37. Zhang R, Brennan ML, Fu X, et al. Association between myeloperoxidase levels and risk of coronary artery disease. *JAMA*. 2001;286(17):2136-2142.

38. Brennan ML, Penn MS, Van Lente F, et al. Prognostic value of myeloperoxidase in patients with chest pain. *N Engl J Med*. 2003;349(17):1595-1604.

39. Asselbergs FW, Tervaert JW, Tio RA. Prognostic value of myeloperoxidase in patients with chest pain. *N Engl J Med*. 2004;350(5):516-518; author reply 516-518.

40. Vita JA, Brennan ML, Gokce N, et al. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. *Circulation*. 2004;110(9):1134-1139.

41. Sambola A, Osende J, Hathcock J, et al. Role of risk factors in the modulation of tissue factor activity and blood thrombogenicity. *Circulation*. 2003;107(7):973-977.

42. Moulton KS, Vakili K, Zurakowski D, et al. Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. *Proc Natl Acad Sci USA*. 2003;100(8):4736-4741.

43. Heeschen C, Dimmeler S, Fichtlscherer S, et al. Prognostic value of placental growth factor in patients with acute chest pain. *JAMA*. 2004;291(4):435-441.

44. Lenderink T, Heeschen C, Fichtlscherer S, et al. Elevated placental growth factor levels are associated with adverse outcomes at four-year follow-up in patients with acute coronary syndromes. *J Am Coll Cardiol*. 2006;47(2):307-311.

45. Abi-Younes S, Sauty A, Mach F, et al. The stromal cell-derived factor-1 chemokine is a potent platelet agonist highly expressed in atherosclerotic plaques. *Circ Res*. 2000;86(2):131-138.

46. Heeschen C, Jang JJ, Weis M, et al. Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. *Nat Med*. 2001;7(7):833-839.

47. Heeschen C, Weis M, Aicher A, et al. A novel angiogenic pathway mediated by non-neuronal nicotinic acetylcholine receptors. J *Clin Invest*. 2002;110(4):527-536.

48. Stuber M, Botnar RM, Fischer SE, et al. Preliminary report on in vivo coronary MRA at 3 Tesla in humans. *Magn Reson Med*. 2002;48(3):425-429.

49. MacNeill BD, Jang IK, Bouma BE, et al. Focal and multifocal plaque macrophage distributions in patients with acute and stable presentations of coronary artery disease. *J Am Coll Cardiol*. 2004;44(5):972-979.

50. Cespedes EI, de Korte CL, van der Steen AF. Intraluminal ultrasonic palpation: assessment of local and cross-sectional tissue stiffness. *Ultrasound Med Biol*. 2000;26(3):385-396.

51. Schaar JA, de Korte CL, Mastik F, et al. Three-dimensional palpography of human coronary arteries. Ex vivo validation and in-patient evaluation. *Herz.* 2005;30(2):125-133.

52. Baldewsing RA, Schaar JA, Mastik F, et al. Assessment of vulnerable plaque composition by matching the deformation of a parametric plaque model to measured plaque deformation. *IEEE Trans Med Imaging*. 2005; 24(4):514-528.

53. Schaar JA, De Korte CL, Mastik F, et al. Characterizing vulnerable plaque features with intravascular elastography. *Circulation*. 2003;108(21):2636-2641.

54. Schaar JA, Regar E, Mastik F, et al. Incidence of high-strain patterns in human coronary arteries: assessment with three-dimensional intravascular palpography and correlation with clinical presentation. *Circulation*. 2004;109:2716-2719.

55. Van Mieghem CA, McFadden EP, de Feyter PJ, et al. Noninvasive detection of subclinical coronary atherosclerosis coupled with assessment of changes in plaque characteristics using novel invasive imaging modalities: the Integrated Biomarker and Imaging Study (IBIS). *J Am Coll Cardiol*. 2006;47(6):1134-1142.

56. Goertz DE, Frijlink ME, Tempel D, et al. Contrast harmonic intravascular ultrasound: a feasibility study for vasa vasorum imaging. *Invest Radiol*. 2006;41(8):631-638.

57. Goertz DE, Frijlink ME, de Jong N, et al. Nonlinear intravascular ultrasound contrast imaging. *Euro Interv.* 2006;2:132-142.

58. Goertz DE, van Wamel A, Frijlink ME, et al. Nonlinear imaging of targeted microbubbles with intravascular ultrasound. *Ultrasonics Symposium, 2005 IEEE*. 2005;4:2003- 2006.

Vulnerable Plaque *Clinical Impact of Assessment*

Post-Test

To receive CME credit, participants can visit: www.CMEdiscovery.com/IAVP or fax/mail to ArcMesa Educators (please refer to page 16). Answer the 8 question multiple choice post-test below and complete the program evaluation. A CME certificate will be issued upon successful completion of the post-test with a score of 70% or higher. Credit expires October 23, 2008. No credit will be given past this date.

1. Based on serial angiographic studies that evaluated prior angiograms in patients who had an MI, what percentage of flow-limiting stenoses (>70%) caused myocardial infarction?

   a) About 15%
   b) About 25%
   c) About 50%
   d) About 65%

2. Results from prospective studies have provided the foundation from which the profile of vulnerable plaque was developed.

   a) True
   b) False

3. Which of the features below is NOT associated with vulnerable plaques?

   a) Large lipid core
   b) Large number of inflammatory cells
   c) Abundance, of smooth muscle cells
   d) Thin fibrous cap

4. Which of the following is most likely to be associated with the oxidized LDL?

   a) Increased plasma level of adiponectin
   b) Reduced plasma level of TNF-alpha
   c) Increased plasma F2 isoprostanes
   d) Increased flow-mediated vasodilation

5. Inflammation in the intima is believed to weaken the integrity of the fibrous cap in a vulnerable plaque because inflammatory signals from the intima inhibit new collagen production in smooth muscle cells, so that they are unable to repair and maintain the collagen in the fibrous cap.

   a) True
   b) False

6. Which of the enzymes below is most likely to be associated with plaque rupture?

   a) Myeloperoxidase
   b) MMP-9
   c) eNO synthase
   d) NADPH oxygenase

7. Which of the applications below is associated with Optical Coherence Tomography?

   a) Identify thin fibrous cap
   b) Measure fibrous cap strength
   c) Detect vasa vasorum
   d) Molecular imaging

8. Which of the technologies below was sensitive enough to detect treatment effects at 6 months in ST-elevated MI patients?

   a) Multislice CT
   b) IVUS
   c) Virtual histology
   d) Palpography

Inflammatory Aspects and Detection *of*

Vulnerable Plaque

Clinical Impact of Assessment

Participant Data (PLEASE PRINT CLEARLY)

Required information for proper certification and individual record retention.

First Name________________________________ Last Name____________________________________

Address____

City____ State______________ Zip__________________

Telephone_______________________________________ Fax____________________________________

Specialty__________________________________ Email____

State of License________________________ License #____

VALIDATION:

I certify that I have studied the course materials and have personally completed the course examination.

Please sign for proper CE/CME certification:____

For Office Use Only

Evaluation Infammatory Aspects of Vulnerable Plaque: Clinical Impact of Assessement

Please provide us with your candid evaluation so that we can continue to improve these continuing education materials. We thank you for your comments and appreciate your suggestions for future courses. Please circle a number between 1 and 5 reflecting you satisfaction with this activity. (5 = Excellent; 1= Poor)

1. How informative did you find this CME activity?

   5 4 3 2 1

2. How well did this CME activity meet the learning objectives?

   5 4 3 2 1

3. How would you rate this CME activity overall?

   5 4 3 2 1

4. Please indicate the amount of time you spent participating in this activity:

   Less than 30 minutes 0.5 – 1.0 hour
   1.0 – 1.5 hours 1.5 – 2.0 hours
   More than 2 hours

5. Do you feel this CME activity was unbiased and non-promotional?

   a. Yes
   b. If no, please explain:

   ____

   ____

6. What is one new thing that you learned from this activity?

   ____

   ____

   ____

7. How will you modify your practice performance as a result of participating in this activity?

   ____

   ____

   ____

8. What topics would you like to see in future presentations?

   ____

   ____

   ____

Mail back to: ArcMesa Educators • 951 State Hwy 33 West • Monroe Township, NJ 08831 • or fax back to: 609-630-6110