Clinical Chemistry 54:1
24–38 (2008)

Review

Inflammation in Atherosclerosis: From Vascular Biology to Biomarker Discovery and Risk Prediction

René R. S. Packard and Peter Libby*

Recent investigations of atherosclerosis have focused on inflammation, providing new insight into mechanisms of disease. Inflammatory cytokines involved in vascular inflammation stimulate the generation of endothelial adhesion molecules, proteases, and other mediators, which may enter the circulation in soluble form. These primary cytokines also induce production of the messenger cytokine interleukin-6, which stimulates the liver to increase production of acute-phase reactants such as C-reactive protein. In addition, platelets and adipose tissue can generate inflammatory mediators relevant to atherothrombosis. Despite the irreplaceable utility of plasma lipid profiles in assessment of atherosclerotic risk, these profiles provide an incomplete picture. Indeed, many cardiovascular events occur in individuals with plasma cholesterol concentrations below the National Cholesterol Education Program thresholds of 200 mg/dL for total cholesterol and 130 mg/dL for low-density lipoprotein (LDL) cholesterol. The concept of the involvement of inflammation in atherosclerosis has spurred the discovery and adoption of inflammatory biomarkers for cardiovascular risk prediction. C-reactive protein is currently the best validated inflammatory biomarker; in addition, soluble CD40 ligand, adiponectin, interleukin 18, and matrix metalloproteinase 9 may provide additional information for cardiovascular risk stratification and prediction. This review retraces the biology of atherothrombosis and the evidence supporting the role of inflammatory biomarkers in predicting primary cardiovascular events in this biologic context.

Leducq Center for Cardiovascular Research, Division of Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

* Address correspondence to this author at: Leducq Center for Cardiovascular Research, Division of Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 77 Avenue Louis Pasteur, NRB 7, Boston, MA 02115. e-mail plibby@rics.bwh.harvard.edu.

Received September 5, 2007; accepted October 30, 2007.
Previously published online at DOI: 10.1373/clinchem.2007.097360

In the past several decades, our understanding of the pathogenesis of atherosclerosis has undergone a revolution *(1)*. Previously, physicians thought of atherosclerosis as primarily a plumbing problem. The degree of stenosis on an angiogram and symptoms and signs of ischemia indicating impaired perfusion of target tissues provided the main tools to assess atherosclerosis. The understanding of the pathophysiology of this disease has now entered a new era based on understanding of the biology and a critical reappraisal of the pathobiology of atherothrombosis *(2)*. The modern biological perspective has revealed that thrombotic complications, culminating, for example, in myocardial infarction, do not necessarily result from critical stenoses. We have also come to appreciate that many myocardial infarctions occur in individuals without previous ischemic symptoms or diagnosis. In up to one-half of individuals, the first manifestation of coronary atherosclerosis is sudden death or myocardial infarction unheralded by premonitory symptoms. This shift in our understanding of the disease underscores the need for novel strategies of a priori risk stratification in seemingly well populations *(3, 4)*.

Understanding of the pathophysiology of atherosclerosis traditionally rests on the cholesterol hypothesis *(5)*. Indeed, besides age, cholesterol and LDL concentrations have indubitable value as risk markers for future cardiovascular events. This epidemiologic relationship engendered decades of detailed scrutiny of cholesterol, cholesterol-trafficking lipoproteins, and the cellular and molecular mechanisms of cholesterol metabolism regulation *(6)*. We have gained an enormous appreciation for the role of modified lipoproteins in the pathogenesis of atherosclerosis. The intense focus on cholesterol in the latter half of the 20th century enabled major strides in therapeutics as well as diagnostics. Unraveling the molecular pathways that regulate cholesterol metabolism led to the development of drug therapies that have proved remarkably effective in reducing clinical events in broad categories of individuals.

Despite the important role of cholesterol in atherosclerosis, many individuals who experience myocardial infarction have cholesterol concentrations at or below the National Cholesterol Education Program

thresholds of 200 mg/dL for total cholesterol and 130 mg/dL for LDL cholesterol *(7)*. Currently, many patients who present with acute myocardial infarctions are receiving drug therapy for dyslipidemia, despite LDL concentrations at currently mandated targets or below. This convergence of clinical findings highlights the necessity of improving our ability to predict cardiovascular risk. Cholesterol has fulfilled Koch postulates as a causal factor in atherosclerosis *(8–12)*. Nonetheless, controversy still exists regarding the mechanisms by which high LDL concentrations actually instigate atherosclerosis and its complications *(13)*. A popular formulation, supported by abundant laboratory and clinical data, suggests that LDL modified by oxidation or by glycation evokes an inflammatory response in the artery wall, unleashing many of the biological processes thought to participate in atherosclerosis initiation, progression, and complication *(14)*. Indeed, inflammation in cells involved in atherosclerosis is elicited by many other risk factors associated with atherosclerosis, including cigarette smoking, insulin resistance/diabetes, and hypertension—particularly that mediated by the renin-angiotensin-aldosterone system *(15)*. Thus, the inflammatory pathways involved in both innate and adaptive immune responses appear to transduce many of the traditional and emerging risk factors for atherosclerosis. We review here the concept of inflammation as a pathogenic principle in atherosclerosis. New biological understanding can point the way to novel biomarkers to predict risk of atherosclerotic events beyond the traditional and well-established risk factors.

INITIATION AND DEVELOPMENT OF ATHEROSCLEROTIC LESIONS

Inflammation participates in atherosclerosis from its inception onwards (Figs. 1 and 2). Fatty streaks do not cause symptoms, and may either progress to more complex lesions or involute. Fatty streaks have focal increases in the content of lipoproteins within regions of the intima, where they associate with components of the extracellular matrix such as proteoglycans, slowing their egress. This retention sequesters lipoproteins within the intima, isolating them from plasma antioxidants, thus favoring their oxidative modification *(16–18)*. Oxidatively modified LDL particles comprise an incompletely defined mixture, because both the lipid and protein moieties can undergo oxidative modification. Constituents of such modified lipoprotein particles can induce a local inflammatory response *(19)*.

Endothelial cells (ECs) normally resist leukocyte adhesion. Proinflammatory stimuli, including a diet high in saturated fat, hypercholesterolemia, obesity, hyperglycemia, insulin resistance, hypertension, and smoking, trigger the endothelial expression of adhesion molecules such as P-selectin and vascular cell adhesion molecule-1 (VCAM-1),[1] which mediate the attachment of circulating monocytes and lymphocytes *(20–22)*. Interestingly, atherosclerotic lesions often form at bifurcations of arteries, regions characterized by disturbed blood flow, which reduces the activity of endothelial atheroprotective molecules such as nitric oxide and favors regional VCAM-1 expression *(23)*.

Chemoattractant factors, which include monocyte chemoattractant protein-1 produced by vascular wall cells in response to modified lipoproteins, direct the

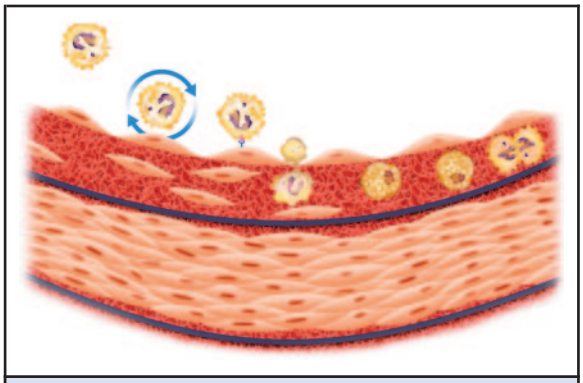

Fig. 1. Initiation of atherosclerosis.

The diagram shows a cross-section through a muscular artery depicting a classic trilaminar structure. The intima of normal arteries is composed of a single layer of endothelial cells overlying a subendothelial matrix that contains occasional resident smooth muscle cells. The underlying tunica media, separated from the intima by the internal elastic lamina, contains multiple layers of vascular smooth muscle cells. The adventitia, the outermost layer of the blood vessel, separated from the media by the external elastic lamina, is not depicted in this diagram. Circulating leukocytes adhere poorly to the normal endothelium under normal conditions. When the endothelium becomes inflamed, however, it expresses adhesion molecules that bind cognate ligands on leukocytes. Selectins mediate a loose rolling interaction of leukocytes with the inflammatorily activated endothelial cells. Integrins mediate firm attachment. Chemokines expressed within atheroma provide a chemotactic stimulus to the adherent leukocytes, directing their diapedesis and migration into the intima, where they take residence and divide. These steps are depicted in a left-to-right chronological sequence. Reprinted with permission from *(91)*.

[1] Nonstandard abbreviations: VCAM-1, vascular cell adhesion molecule-1; MMP-9, matrix metalloproteinase 9; IL, interleukin; IFN, interferon; SMC, smooth muscle cell; PAI-1, plasminogen activator inhibitor 1; MRP, myeloid-related protein; CRP, C-reactive protein; C, cholesterol; hs, high-sensitivity; PPAR, peroxisome proliferator-activated receptor.

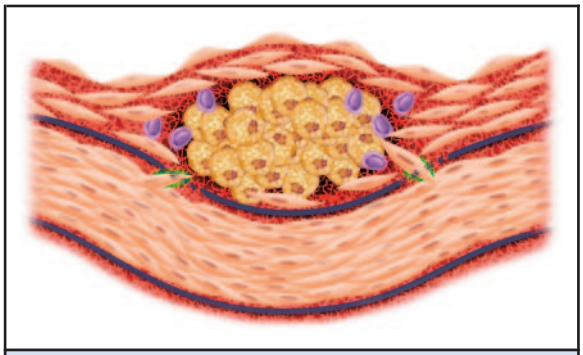

Fig. 2. Progression of atherosclerosis.

Macrophages augment the expression of scavenger receptors in response to inflammatory mediators, transforming them into lipid-laden foam cells following the endocytosis of modified lipoprotein particles. Macrophage-derived foam cells drive lesion progression by secreting proinflammatory cytokines. T lymphocytes join macrophages in the intima and direct adaptive immune responses. These leukocytes, as well as endothelial cells, secrete additional cytokines and growth factors that promote the migration and proliferation of SMCs. In response to inflammatory stimulation, vascular SMCs express specialized enzymes that can degrade elastin and collagen, allowing their penetration into the expanding lesion. Reprinted with permission from *(91)*.

migration and diapedesis of adherent monocytes *(24, 25)*. Monocytic cells directly interacting with human ECs increase monocyte matrix metalloproteinase 9 (MMP-9) production several fold, allowing for the subsequent infiltration of leukocytes through the endothelial layer and its associated basement membrane *(26)*. Within the intima, monocytes mature into macrophages under the influence of macrophage colony-stimulating factor, which is overexpressed in the inflamed intima *(27, 28)*. Macrophage colony-stimulating factor stimulation also increases macrophage expression of scavenger receptors, members of the pattern-recognition receptor superfamily, which engulf modified lipoproteins through receptor-mediated endocytosis. Accumulation of cholesteryl esters in the cytoplasm converts macrophages into foam cells, i.e., lipid-laden macrophages characteristic of early-stage atherosclerosis. In parallel, macrophages proliferate and amplify the inflammatory response through the secretion of numerous growth factors and cytokines, including tumor necrosis factor α and interleukin (IL)-1β. Recent evidence supports selective recruitment of a proinflammatory subset of monocytes to nascent atheroma in mice *(29, 30)*. These observations point to a previously unappreciated layer of complexity in the inflammatory aspects of early atherogenesis.

T cells, representing the adaptive arm of the immune response, also play a critical role in atherogenesis, entering lesions in response to the chemokine-inducible protein-10, monokine induced by interferon (IFN)-γ, and IFN-inducible T cell α-chemoattractant *(31)*. The CD4+ subtype, which recognizes antigens presented as fragments bound to major histocompatibility complex class II molecules, predominates in the lesion. Interestingly, human lesions contain CD4+ T cells reactive to the disease-related antigens associated with oxidized LDL *(32)*. The atherosclerotic lesion contains cytokines that promote a T-helper 1 response, inducing activated T cells to differentiate into T-helper 1 effector cells *(33)*. These cells amplify the local inflammatory activity by producing proinflammatory cytokines such as IFN-γ and CD40 ligand (CD40L, CD154), which contribute importantly to plaque progression.

Adiponectin, a product of adipose tissue, has insulin-sensitizing, antiatherogenic, and antiinflammatory properties *(34)*. An important autocrine/paracrine factor in adipose tissue, it modulates the differentiation of preadipocytes and favors the formation of mature adipocytes. Curiously, adiponectin concentrations are lower in obese than lean individuals. This adipokine also functions as an endocrine factor, influencing whole-body metabolism via effects on target organs. Adiponectin exerts multiple biologic effects pivotal to cardiovascular biology, including increasing insulin sensitivity, reducing visceral adipose mass, reducing plasma triglycerides, and increasing high-density lipoprotein (HDL) cholesterol *(35)*. Adiponectin alters the concentrations and activity of enzymes responsible for the catabolism of triglyceride-rich lipoproteins and HDL, such as lipoprotein lipase and hepatic lipase. It thus influences atherosclerosis by affecting the balance of atherogenic and antiatherogenic lipoproteins in plasma *(36)*. Adiponectin also directly affects the function of endothelial cells, reducing VCAM-1 expression, and macrophages, decreasing the expression of scavenger receptors and the production of tumor necrosis factor α *(34, 37)*.

PROGRESSION TO COMPLEX ATHEROSCLEROTIC LESIONS

Macrophages and T cells infiltrate atherosclerotic lesions and localize particularly in the shoulder region, where the atheroma grows. Whereas foam cell accumulation characterizes fatty streaks, deposition of fibrous tissue defines the more advanced atherosclerotic lesion. Smooth muscle cells (SMCs) synthesize the bulk of the extracellular matrix that characterizes this phase of plaque evolution *(38)*. In response to platelet-derived growth factor released by activated macrophages and endothelial cells, and silent plaque disruptions that lead to clinically unapparent mural thrombi, SMCs migrate from the tunica media into the intima

via degradation of the extracellular matrix mediated by MMP-9 and other proteinases *(39)*. In the intima, SMCs proliferate under the influence of various growth factors and secrete extracellular matrix proteins, including interstitial collagen, especially in response to transforming growth factor-β and platelet-derived growth factor. This process causes the lesion to evolve from a lipid-rich plaque to a fibrotic and, ultimately, a calcified plaque that may create a stenosis.

Human atheromata express IL-18 and increased concentrations of its receptor subunits, IL-18Rα/β *(40)*. IL-18 occurs predominantly as the mature 18-kD form and colocalizes with mononuclear phagocytes while ECs, SMCs, and macrophages all express IL-18Rα/β. Importantly, IL-18 signaling evokes essential effectors involved in atherogenesis, e.g., adhesion molecules (VCAM-1), chemokines (IL-8), cytokines (IL-6), and matrix metalloproteinases (MMP-1/-9/-13). In addition, IL-18, particularly in combination with IL-12, is a proximal inducer and regulator of the expression of IFN-γ, a major proinflammatory cytokine, during atherogenesis. Interestingly, IL-18 induces IFN-γ expression not only in T cells *(41)*, but also in macrophages and, surprisingly, even in SMCs, thus activating in a paracrine mode several proinflammatory pathways operating during atherogenesis *(40)*.

Neovascularization arising from the artery's vasa vasorum contributes to lesion progression in many ways *(42)*. It provides another portal for leukocyte entry into established atherosclerotic lesions *(43)*. In addition, these fragile neovessels can favor focal intraplaque hemorrhage that provides a mechanism for the discontinuous increments seen in plaque growth. Local hemorrhage within the plaque in turn generates thrombin, which activates ECs, monocytes/macrophages, SMCs, and platelets *(44)*. These cells respond to thrombin by producing a broad array of inflammatory mediators, including CD40L, RANTES (regulated on activation, normal T cell expressed and secreted), and macrophage migration inhibitory factor. These molecules further promote lesion formation and favor the thrombotic complications of atherosclerosis *(45)*. Platelets also play a central role in the biology of atherosclerosis by producing inflammatory mediators such as CD40L, myeloid-related protein-8/14, and platelet-derived growth factor, as well as directing leukocyte incorporation into plaques through platelet-mediated leukocyte adhesion. These results reveal the synergism between inflammation and thrombosis in the pathobiology of atherothrombosis *(44)*.

CD40L plays an important role in this phase of atherogenesis. All the main cell types involved in atherosclerosis, including ECs, macrophages, T cells, SMCs, and platelets, express this proinflammatory cytokine as well as its receptor, CD40 *(46)*. CD40 ligation triggers the expression of adhesion molecules and the secretion of numerous cytokines and MMPs involved in extracellular matrix degradation *(47–49)*. Importantly, CD40L has a prothrombotic effect, inducing EC *(50)*, macrophage *(47)*, and SMC *(51)* expression of tissue factor, which initiates the coagulation cascade when exposed to factor VII. Accordingly, inhibition of CD40 signaling reduces experimental atherosclerosis development *(52)* as well as the evolution of established atherosclerosis *(53)*.

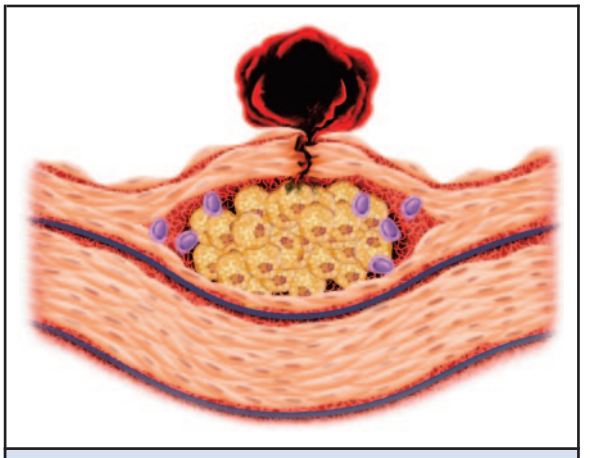

Fig. 3. Thrombotic complication of atherosclerosis.
Ultimately, inflammatory mediators can inhibit collagen synthesis and evoke the expression of collagenases by macrophage foam cells within the intima. This imbalance diminishes the collagen content of the fibrous cap, rendering it weak and rupture-prone. In parallel, crosstalk between T lymphocytes and other cell types present within lesions heightens the expression of the potent procoagulant tissue factor. Thus, when the fibrous cap ruptures, as illustrated in this diagram, tissue factor induced by inflammatory signaling triggers the thrombus that causes most acute complications of atherosclerosis. Clinically, this may translate into an acute coronary syndrome. Reprinted with permission from *(91)*.

PLAQUE RUPTURE AND PATHOGENESIS OF ACUTE CORONARY SYNDROMES

Plaque rupture and the ensuing thrombosis commonly cause the most dreaded acute complications of atherosclerosis (Fig. 3). In many cases, the culprit lesion of acute coronary artery thrombosis does not produce a critical arterial narrowing, rendering its a priori identification using standard angiographic methods uncertain *(54)*. Indeed, it now appears that inflammatory activation, rather than the degree of stenosis, renders the plaque rupture prone and precipitates thrombosis and resulting tissue ischemia *(55)*. Advanced complex atheromata exhibit a paucity of SMCs at sites of rup-

ture and abundant macrophages, key histological characteristics of plaques that have ruptured and caused fatal coronary thrombosis. Inflammation can interfere with the integrity of the interstitial collagen of the fibrous cap by stimulating the destruction of existing collagen fibers and by blocking the creation of new collagen *(56)*. IFN-γ, secreted by activated T cells, inhibits collagen production by SMCs. T lymphocytes can also contribute to the control of collagenolysis. CD40L as well as IL-1 produced by T cells induce macrophages to release interstitial collagenases, including MMP-1, -8, and -13 *(57)*. The shoulder region of plaques as well as areas of foam cell accumulation contain MMP-9, a member of the gelatinase class of the metalloproteinase family *(58)*. Interestingly, retroviral overexpression of an active form of MMP-9 in macrophages induces morphologic appearances interpreted as plaque disruption *(59)*. Human plaque analysis has revealed that MMP-9 is catalytically active and may thus contribute to the dysregulation of extracellular matrix that leads to plaque rupture during the complication of atherothrombosis *(58)*. Further evidence suggests that local overexpression of MMP-9 promotes intravascular thrombus formation through increased tissue factor expression and tissue factor–mediated activation of the coagulation cascade *(60)*. These data support an important role for MMP-9 in several stages of atherosclerosis.

Acute coronary syndromes most often result from a physical disruption of the fibrous cap, either frank cap fracture or superficial endothelial erosion, allowing the blood to make contact with the thrombogenic material in the lipid core or the subendothelial region of the intima *(55)*. This contact initiates the formation of a thrombus, which can lead to a sudden and dramatic obstruction of blood flow through the affected artery. If the thrombus is nonocclusive or transient, it may either be clinically silent or cause symptoms characteristic of an acute coronary syndrome. Importantly, with relation to the propensity of a given plaque disruption to lead to a sustained and occlusive thrombus, the fluid phase of blood, most notably circulating plasminogen activator inhibitor 1 (PAI-1) and fibrinogen concentrations, may determine the fate of a given plaque disruption *(1, 61, 62)*. Indeed, impaired fibrinolysis can result from an imbalance between clot-dissolving enzymes and their endogenous inhibitors, primarily PAI-1 *(63)*. PAI-1 belongs to the serine protease inhibitor superfamily (serpins) and originates from several sites, including the endothelium, liver, and adipose tissue *(64)*. Experimental work using transgenic mice that overexpress a stable form of human PAI-1 demonstrates an association of chronically increased concentrations of PAI-1 with age-dependent coronary arterial thrombosis *(65)*.

The foregoing discussion illustrates the principle of inflammatory mediator involvement in atherosclerosis with examples drawn from the authors' own experiments. The multiplicity of mediators implicated in atherogenesis and the plenitude of potential biomarkers of disease by far exceed the scope of this review. Interested readers can consult other authoritative compilations *(66–72)*.

In summary, inflammation participates pivotally in all stages of atherosclerosis, from lesion initiation to progression and destabilization. In addition, inflammation regulates both the "solid-state" thrombotic potential in the plaque itself and the prothrombotic and antifibrinolytic capacity of blood in the fluid phase *(1)*. The ominous presence of inflammation in atherosclerosis has prompted the evaluation of certain key inflammatory factors in cardiovascular risk prediction.

TECHNICAL CONSIDERATIONS OF BIOMARKERS

Given this new understanding of the central function of inflammation in atherogenesis, could inflammatory biomarkers, independent of cholesterol and regulators of blood pressure, further report on the different aspects of the pathogenic mechanisms that underlie this disease? To provide a framework for this discussion, with Paul M. Ridker we have proposed a proinflammatory pathway at work during atherogenesis (Fig. 4).

Biomarkers of inflammation include adhesion molecules such as VCAM-1; cytokines such as tumor necrosis factor, IL-1, and IL-18; proteases such as MMP-9; the messenger cytokine IL-6; platelet products including CD40L and myeloid-related protein (MRP) 8/14; adipokines such as adiponectin; and finally, acute phase reactants such as C-reactive protein (CRP), PAI-1, and fibrinogen.

With regard to clinical utility, one must ask a number of questions regarding putative biomarkers of cardiovascular risk. Does the marker add information to that available from existing and well-established risk factors? Is the marker a suitable analyte? Is the marker stable with respect to diet and time of day, as well as from day to day? Ideally, a proposed biomarker should not only provide independent information on cardiovascular risk, but also be easy to measure using inexpensive and standardized commercial assays with low variability that do not require specialized plasma collection or assay techniques.

In this regard CRP has proved most robust, as it is an excellent analyte with a standardized assay, has negligible diurnal variation, does not depend on food intake, and has a long half-life, in addition to a remarkable dynamic range. It is easily measured, and standardized high-sensitivity immunoassays (detecting CRP concentrations $<$10 mg/L) provide similar results in fresh, stored, or frozen plasma, reflecting the stabil-

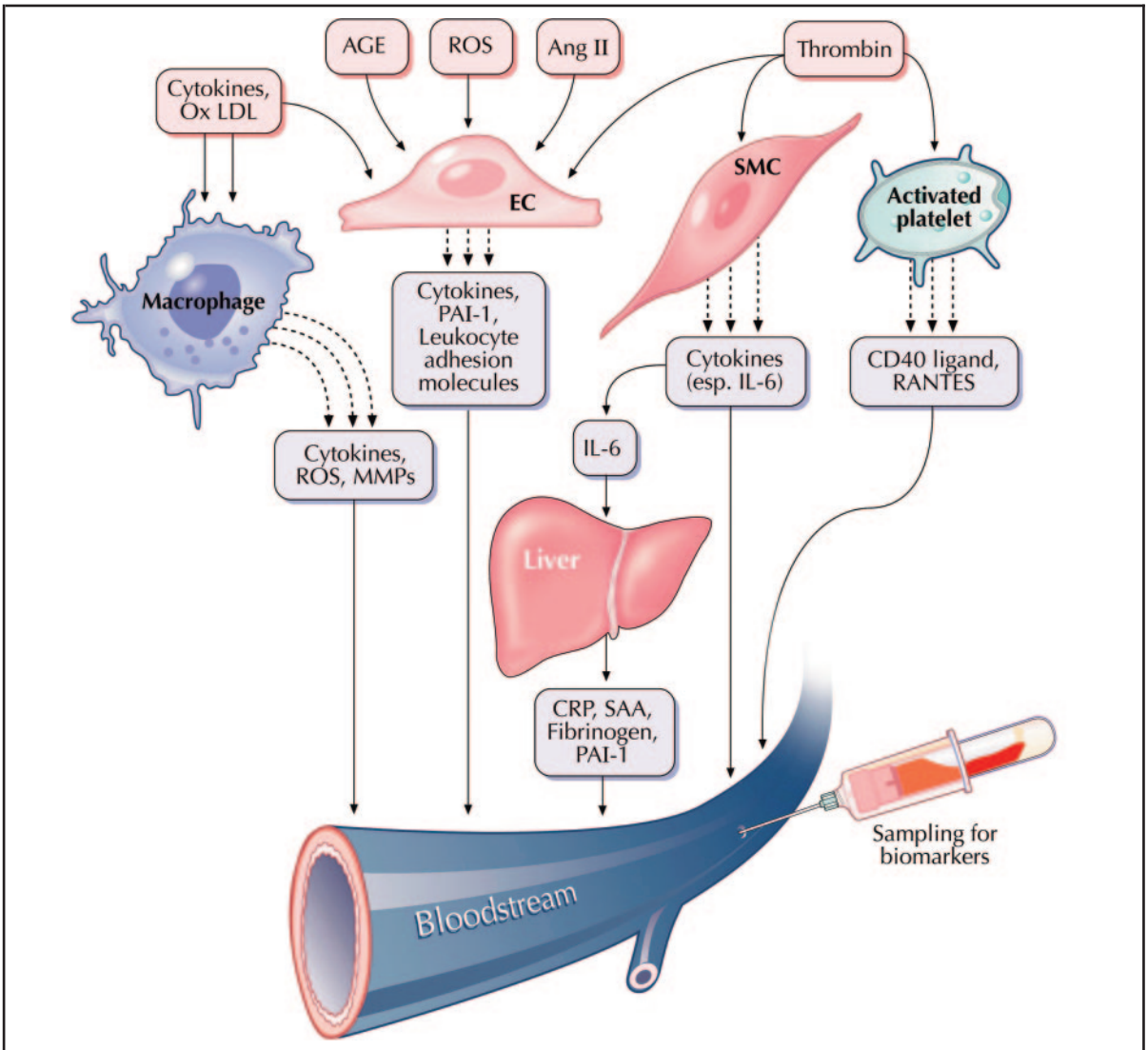

Fig. 4. Inflammation links classic risk factors to altered cellular behavior within the arterial wall and secretion of inflammatory markers in the circulation.

Primary proinflammatory risk factors elicit the expression of primary proinflammatory cytokines that can be released directly into the blood. Cytokines orchestrate the production of adhesion molecules, matrix metalloproteinases, and reactive oxygen species that may also be released from lesions. In parallel, these primary cytokines induce the expression of the messenger cytokine IL-6, particularly in smooth muscle cells. IL-6 then travels to the liver, where it elicits the acute-phase response, resulting in the release of C-reactive protein, fibrinogen, and plasminogen activator inhibitor-1. All these inflammatory markers and mediators, released at different stages in the pathobiology of atherothrombosis, can enter the circulation, where they can be easily measured in a peripheral vein. *AGE*, advanced glycation end products; *Ang II*, angiotensin II; *OxLDL*, oxidized low-density lipoprotein; *RANTES*, regulated on activation, normal T cell expressed and secreted; *ROS*, reactive oxygen species; *SAA*, serum amyloid A. Reprinted with permission from *(162)*.

ity of the protein, which has led CRP to emerge as a robust *(73)* (albeit controversial *(74–76)*) clinical marker. Other acute-phase reactants, such as PAI-1 and fibrinogen, clearly participate in the pathogenic pathway of atherothrombosis. They appear less useful than CRP, however, because of their muted dynamic range. PAI-1 circulates with a half-life of 6 min and displays a circadian variation. In addition, to measure PAI-1 accurately one must draw blood meticulously and process samples rapidly, precautions that are not

practical in the clinic. Fibrinogen has a diurnal variation, and its reproducible measurement and standardization has proved challenging. Adiponectin has minimal diurnal variation, making it more suitable for clinical analysis. Other mediators such as IL-1 may have great biological basis for potential use as biomarkers but have short half-lives that confound their benefit in routine risk prediction. IL-6 is not readily measured in clinical settings, partly due to its short half-life in plasma.

BIOMARKERS VS MEDIATORS OF DISEASE

The distinction between biomarkers vs mediators of disease has proven quite confusing. As discussed in examples above, a particular analyte may participate clearly in a pathogenic pathway but not serve as an effective biomarker.

Soluble VCAM-1, for example, does not predict the risk of future myocardial infarction in apparently healthy men *(77)*. However, research has repeatedly and unequivocally demonstrated the essential role of VCAM-1 in experimental atherosclerotic lesion initiation and progression *(20, 21, 78–81)*.

On the other hand, a useful biomarker may not mediate pathogenic processes associated with disease. In the case of CRP, the bulk of current evidence supports its utility as a biomarker of risk, not only in apparently healthy populations but also in risk stratification of individuals with established disease. Yet, the role of CRP as a mediator rests on a less secure foundation; notably, many in vitro studies with CRP have used extraordinarily high concentrations of the molecule, causing concern about endotoxin contamination or preservatives in CRP preparations that might have spurious effects on cells. Experimental results suggest that CRP displays a direct proinflammatory effect on endothelial cells *(82)* and mediates LDL uptake by macrophages *(83)*. In vivo data, both in animals and humans, suggest that CRP may promote processes involved in the pathogenesis of atherothrombosis, including dysregulation of fibrinolysis by increasing the expression and activity of PAI-1 *(84)*. Recent studies have also shown that CRP originates not only in the liver, but also from other tissues, including SMCs from normal coronary arteries *(85)* and diseased coronary artery bypass grafts *(86)* as well as coronary artery endothelial cells *(87)*, which may provide an explanation for potential local actions of CRP.

RATIONALE FOR NOVEL BIOMARKERS OF CARDIOVASCULAR RISK PREDICTION

The only blood biomarkers currently recommended for use in cardiovascular risk prediction by the Adult Treatment Panel are LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglycerides *(88)*. However, plasma total cholesterol concentrations alone poorly discriminate risk for coronary heart disease, as more than half of all vascular events occur in individuals with below-average total cholesterol concentrations *(7, 89)*. As our understanding of the biology of atherothrombosis has improved *(55, 90, 2)*, evaluation has commenced of a series of candidate biomarkers reflecting inflammation, oxidative stress, and thrombosis as potential clinical tools for improving risk prediction *(91, 4, 92)*.

Although global risk assessment offers improved prediction of cardiovascular events *(93)*, emerging data suggest that measurement of inflammatory markers may enhance risk evaluation. Current evidence suggests a pathway of inflammation in atherosclerosis that culminates in altered concentrations of various markers in peripheral blood *(3)*. This review focuses specifically on established and emerging inflammatory biomarkers involved in the prediction of a primary cardiovascular event. In particular, beyond CRP, sCD40L, adiponectin, IL-18, and MMP-9 warrant special emphasis as inflammatory biomarkers at least as research tools, if not currently appropriate for routine clinical use. We will not discuss here markers involved in primary prediction and risk stratification that are not measurable in blood/plasma/serum by ELISA (e.g., emerging molecular imaging modalities, interventional techniques), or markers involved in risk stratification at the time of an acute coronary syndrome for the secondary prevention of cardiovascular disease. Markers of oxidative stress, LDL oxidation, and heart failure are treated elsewhere.

ROLE OF ESTABLISHED AND EMERGING INFLAMMATORY BIOMARKERS IN CARDIOVASCULAR RISK ASSESSMENT

C-REACTIVE PROTEIN

Data from multiple large-scale prospective studies demonstrate that CRP strongly and independently predicts adverse cardiovascular events, including myocardial infarction, ischemic stroke, and sudden cardiac death *(89, 94–98)*. Indeed, with increasing levels of adverse cardiovascular events, baseline concentrations of CRP follow a parallel and graded rise. The addition of CRP to traditional cholesterol screening enhances cardiovascular risk prediction independently of LDL-C, suggesting that increased CRP concentrations in particular may identify asymptomatic individuals with average cholesterol concentrations at high risk for future cardiovascular events *(89)*. Concentrations of CRP add important prognostic information on cardiovascular risk not only at all concentrations of LDL-C but also at all levels of the Framingham risk score *(89, 97)*.

In addition, components of the metabolic syndrome (i.e., central obesity, increased plasma triglyc-

eride concentrations, low plasma concentrations of HDL-C, hypertension, and increased concentrations of blood glucose) correlate with increased plasma CRP concentrations *(89)*, and CRP measurement contributes to risk prediction in individuals with the metabolic syndrome *(99)*.

These results have led to the development of the Reynolds risk score in an effort to ameliorate the assessment of global cardiovascular risk in women *(100)*. This algorithm adds CRP to the Framingham risk score and improves global cardiovascular risk prediction by correctly reclassifying up to 50% of women deemed at intermediate risk into higher- or lower-risk categories.

Based on these data and to improve cardiovascular risk stratification in primary prevention populations, an expert panel assembled by the Centers for Disease Control and Prevention and the American Heart Association termed CRP an independent marker of cardiovascular risk *(101)*. The panel recommends the use of CRP as part of global risk prediction in asymptomatic individuals, particularly those deemed at intermediate risk for cardiovascular disease by traditional risk factors. The recommended cutoff points in clinical practice are CRP concentrations <1 mg/L for low-risk and >3 mg/L for high-risk individuals.

The magnitude of risk reduction associated with statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) exceeds that predicted on the basis of LDL-C lowering alone. The Cholesterol and Recurrent Events (CARE) trial first demonstrated that statin therapy lowers plasma concentrations of CRP in addition to LDL-C *(102)*. Evidence shows this effect holds across the class, with statin therapy reducing CRP concentrations approximately 20% to 30%.

Future investigations will determine whether plasma CRP measurement can identify individuals who, while apparently at low risk, may still benefit from lipid-lowering therapy. Retrospective evidence supports this hypothesis. The Air Force/Texas Coronary Atherosclerosis Prevention Study included men and women without coronary heart disease who had average total and LDL-C plasma concentrations and below-average HDL-C plasma concentrations. Compared with results of the placebo arm, the statin arm demonstrated marked event reduction in the patients with above median plasma total cholesterol:HDL-C ratio and/or plasma CRP, and even in those individuals with below median total cholesterol:HDL-C ratio but above median CRP. In contrast, statin therapy had little effect on the rate of events in individuals with low ratio and low CRP values. These results suggested that statin therapy may prevent coronary events among persons with relatively low lipid concentrations but slightly increased CRP concentrations *(103)*.

A prespecified analysis of the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE-IT TIMI 22) trial revealed similar associations between CRP reduction and risk of recurrent coronary events among patients with acute coronary syndromes *(104)*. When divided into categories based on final CRP and LDL-C concentrations achieved, patients with low CRP concentrations after statin therapy had better clinical outcomes than those with high CRP concentrations, regardless of resulting LDL-C concentrations.

Moreover, each trial found only a small correlation in individual participants between CRP reduction and LDL-C reduction achieved with statin use (correlation coefficient, 0.1–0.2).

The Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial demonstrated that lowering CRP concentrations in patients with coronary disease with intensive statin therapy results in reduced atherosclerotic lesion progression *(105)*.

Together these results suggest that measurement of CRP in addition to LDL-C concentrations may inform the primary and secondary prevention of cardiovascular disease. In this regard the critical question has emerged whether CRP can indicate which patients will benefit from statin therapy despite having "average" LDL-C values. Definitive prospective evidence for a broader application in cardiovascular event reduction remains undetermined. A large-scale, randomized clinical trial, JUPITER (Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin), will evaluate the effects of statin therapy in individuals who have both plasma LDL-C concentrations below those currently used to target therapy and plasma CRP concentrations that indicate heightened risk of a cardiovascular event *(106)*. This investigation will clarify whether monitoring CRP is beneficial for primary prevention.

FIBRINOGEN

Several large-scale epidemiologic studies demonstrate that baseline fibrinogen concentrations predict future risk of myocardial infarction and stroke *(107–109)*. When compared head-to-head with CRP, fibrinogen seems a less potent predictor of cardiovascular events *(110)*. Illustrating the importance of detection methodology, when fibrinogen is measured with a reliable and high-quality immunoassay, there is a significant association between higher concentrations of fibrinogen and CRP, alone and in combination, and incident cardiovascular disease in apparently healthy women over a 10-year follow-up period *(111)*.

PLASMINOGEN ACTIVATOR INHIBITOR 1

PAI-1 circulates with a half-life of 6 min. A number of essential cardiovascular risk factors, including genetic predisposition *(112*, *113)*, insulin resistance *(114)*, and neurohormonal factors *(115)*, directly influence PAI-1 production. Accordingly, PAI-1 may furnish a composite indication of inflammation, metabolic control, and neurohormonal activation, all of which may contribute either independently or synergistically to cardiovascular disease. Increased concentrations of PAI-1 predict the occurrence of a first acute myocardial infarction in middle-aged men and women with a high prevalence of coronary heart disease *(116)*.

Interestingly, certain agents known to reduce vascular risk modify PAI-1 concentrations. Most importantly, numerous studies indicate that angiotensin-converting enzyme inhibitors decrease PAI-1 concentrations across different ethnic groups in both primary *(117)* and secondary *(118)* prevention.

In addition to practical considerations limiting its use, the independence of the predictive value of PAI-1 from traditional risk factors remains questionable. Currently, no evidence shows that knowledge of baseline PAI-1 concentrations adds prognostic information to Framingham risk scoring.

SOLUBLE CD40 LIGAND

Preanalytical sampling conditions critically influence sCD40L concentration, and only plasma samples are appropriate for sCD40L measurements *(119*, *120)*.

Interestingly, the metabolic syndrome as defined by the National Cholesterol Education Program associates independently with increased sCD40L concentrations in multivariate analysis *(121)*.

Results from the Dallas Heart Study suggest that sCD40L does not identify subclinical atherosclerosis in the general population *(122)*. In patients with stable coronary artery disease documented by angiography, however, an association is evident between atheroma burden, stenosis, and abnormally increased circulating concentrations of sCD40L *(123)*. In addition, patients with evidence of a lipid pool on high-resolution magnetic resonance imaging of carotid stenoses have increased sCD40L concentrations *(124)*.

Results from the Women's Health Study suggest that high plasma concentrations of sCD40L associate with increased vascular risk in apparently healthy women *(125)*. Indeed, mean concentrations of sCD40L at baseline were significantly higher among participants who subsequently developed myocardial infarction, stroke, or cardiovascular death compared with age- and smoking-matched women who remained free of cardiovascular disease during a 4-year follow-up. In particular, women with concentrations above the 95th percentile of the control distribution had a significantly increased relative risk of 2.8 of developing future cardiovascular events after adjustment for usual cardiovascular risk factors *(125)*. In asymptomatic patients with low-grade carotid stenosis, increased sCD40L concentrations predict the risk of an adverse cardiovascular event *(126)*. In patients with end-stage renal disease on hemodialysis, increased concentrations of sCD40L strongly and independently predict (relative risk 6.8) nonfatal and fatal atherothrombotic events *(127)*.

Importantly, sCD40L elevation identifies a subgroup of patients at high risk for an adverse cardiovascular event who would likely benefit from antiplatelet treatment through glycoprotein IIb/IIIa receptor inhibition with abciximab *(128)*. In addition, in patients with acute coronary syndromes, atorvastatin abrogates the risk of recurrent cardiovascular events associated with high sCD40L by 48% while only modestly decreasing sCD40L concentrations *(129)*.

MYELOID-RELATED PROTEIN 8/14

Platelets and macrophages release most MRP-14, which heterodimerizes with MRP-8. Its expression increases before ST-elevation myocardial infarction, and increasing plasma concentrations of MRP-8/14 among healthy individuals predict the risk of future cardiovascular events *(130)*. Patients with the highest concentrations have a 3.8-fold increase in risk of experiencing a vascular event, independent of yet additive to standard cardiovascular risk factors and CRP.

ADIPONECTIN

Adiponectin has insulin-sensitizing effects, and secretion of adiponectin diminishes as adipose tissue mass increases. As such, obese adult patients with type 2 diabetes mellitus, essential hypertension, dyslipidemia, and cardiovascular disease have reduced adiponectin concentrations compared to a healthy lean population *(35)*.

Experimental and clinical evidence suggest that adiponectin contributes to the relationship between obesity, insulin resistance, and cardiovascular disease. Adiponectin concentrations inversely correlate with the presence of components of the metabolic syndrome *(131)*. Importantly, men have substantially lower concentrations of adiponectin than premenopausal women. Low concentrations of adiponectin associate with insulin resistance, visceral adiposity, and related metabolic syndrome, and also with positive parental histories of coronary heart disease, hypertension, and type 2 diabetes mellitus, underscoring the value of adiponectin in cardiovascular and type 2 diabetes mellitus risk assessments in young adults *(132)*. In obese premenopausal women (body mass index $\geq$30 kg/m^2) without diabetes, hypertension, or hyperlipidemia, losing at least 10% of body weight through

a low-energy Mediterranean-style diet and increased physical activity decreased body mass index while significantly increasing adiponectin concentrations *(133)*. These observations suggest that adiponectin concentrations change dynamically and respond inversely to changes in metabolic status. In a 5-year prospective study, low baseline serum adiponectin concentrations significantly and independently predicted incident hypertension (defined as a sitting blood pressure ≥140/90 mmHg) in a nondiabetic patient cohort *(134)*.

In one study, adiponectin concentrations in healthy middle-aged subjects independently and negatively associated with carotid artery intima-media thickness *(135)*. Another study demonstrated that low plasma adiponectin concentrations correlated with increased carotid artery intima-media thickness—this time in male patients with type 2 diabetes mellitus—independently of conventional cardiovascular risk factors, insulin resistance, and plasma CRP concentrations *(136)*. Men and women with angiographically confirmed stable coronary artery disease have lower adiponectin serum concentrations compared with age- and sex-matched controls *(137)*. A subsequent study confirmed these results across ethnic groups, with subjects suffering from coronary artery disease having lower concentrations of adiponectin than those free of disease *(138)*. Finally, plasma adiponectin concentrations associate inversely with the extent and complexity (defined as stenosis with irregular, rough borders; ulcerations; and long atherosclerotic lesions with severe narrowing) of coronary lesions determined by angiography in men with coronary artery disease *(139)*. These results suggest that low adiponectin concentrations contribute to a less fibrous and more rupture-prone coronary plaque character.

In a nested case-control study involving participants of the Health Professionals Follow-up Study (18 225 men ages 40–75 and free of diagnosed cardiovascular disease at the time of blood draw), researchers prospectively assessed baseline plasma adiponectin concentrations for associated risk of myocardial infarction during 6 years of follow-up. After adjustment for matched variables, participants in the highest compared with the lowest quintile of adiponectin concentrations had a significantly decreased risk of myocardial infarction (relative risk = 0.39; P for trend <.001) *(140)*. In an 18-year follow-up of apparently healthy middle-aged men, measuring adiponectin concentrations in patients with low HDL values identifies individuals at very high risk for type 2 diabetes and adverse cardiovascular events *(141)*. A low concentration of adiponectin is also a significant risk factor for the development of adverse cardiovascular events in patients with type 2 diabetes *(142)* as well as those with end-stage renal disease *(143)*.

Recent results have cast some doubt on the consistency of the inverse association between adiponectin concentrations and cardiovascular risk. In a 20-year prospective analysis, baseline adiponectin did not associate with fatal cardiovascular events *(144)*. A recent prospective study of 4046 men ages 60–79 followed up for a mean of 6 years suggests that in this patient category, high adiponectin concentrations associate with increased all-cause and cardiovascular mortality regardless of the presence or absence of underlying cardiovascular disease *(145)*. These results suggest the need for additional studies to determine the utility of adiponectin in cardiovascular risk stratification.

The adiponectin gene promoter region has peroxisome proliferator response elements, suggesting that peroxisome proliferator-activated receptor (PPAR) ligands increase adiponectin. Indeed, bezafibrate (PPAR-α ligand)-treated subjects had increased serum adiponectin, compared with the placebo group *(146)*. Higher adiponectin concentrations strongly associated with reduced risk of new diabetes, suggesting that fibrates enhance adiponectin partly through adipose tissue PPAR-α activation and that measurement of adiponectin would be a useful tool for evaluating subjects at high risk for diabetes *(146)*. In nondiabetic subjects with low HDL-C concentrations, rosiglitazone—a thiazolidinedione (PPAR-γ ligand)—significantly increases adiponectin concentrations without significantly affecting HDL-C concentrations *(147)*. Another thiazolidinedione—pioglitazone—in combination with simvastatin also significantly increases adiponectin concentrations in a nondiabetic population at cardiovascular risk *(148)*.

INTERLEUKIN-18

Human preadipocytes of all differentiation stages spontaneously secrete IL-18, supporting the concept that adipocytes participate in innate immunity and that IL-18 mediates a fraction of the complications of obesity such as cardiovascular disease and type 2 diabetes *(149)*. Importantly, IL-18 release from adipocytes of obese patients exceeds by some 3-fold that from adipocytes of nonobese donors *(149)*. Increased concentrations of IL-18 associate with a significantly increased risk of developing type 2 diabetes in middle-aged men and women after adjustment for classic risk factors such as age, body mass index, systolic blood pressure, and physical activity *(150)*. In addition, IL-18 may predict development of the metabolic syndrome, with concentrations rising in parallel to increasing numbers of metabolic risk factors *(151)*.

IL-18 is not currently considered a useful screening tool for the presence of subclinical atherosclerosis in the general population, on the basis of results from 2 large independent imaging studies *(152, 153)*. How-

ever, in the AtheroGene study, IL-18 serum concentration independently predicted cardiovascular death in patients with documented coronary artery disease *(154)*. In this patient population, those within the highest quartile of IL-18 had a 3.3-fold increase in hazard risk compared to those in the first quartile *(154)*. In addition, data from the Prospective Epidemiological Study of Myocardial Infarction (PRIME) demonstrate an independent association between baseline plasma IL-18 concentration in healthy middle-aged men and future coronary events *(155)*. This association remains after adjustment for classic cardiovascular risk factors. These studies suggest that IL-18 measurement may add prognostic information to lipid and inflammatory markers in patients with or without clinically established atherosclerotic disease.

MATRIX METALLOPROTEINASE 9

Aortic stiffness—an independent determinant of cardiovascular risk—relates positively to circulating MMP-9 concentrations, suggesting a role for this elastin-degrading enzyme in the development of systolic hypertension *(156)*.

Patients with stable coronary artery disease have higher circulating concentrations of MMP-9 than healthy controls *(123)*. In addition, individuals with angiographically documented stable coronary artery disease have increased MMP-9 serum concentrations compared to controls, and MMP-9 correlates positively with LDL-C concentrations and negatively with HDL-C concentrations *(157)*.

Plasma MMP-9 concentrations during acute coronary syndromes are increased 2- to 3-fold compared to normal *(158, 159)*. Within a week, the initial MMP-9 elevation reverses back toward the control range, supporting an active role for MMP-9 in the pathogenesis of plaque rupture *(158)*.

In a prospective study of patients followed for a mean of 4.4 years, increased baseline concentrations of MMP-9 in subjects with ≥50% carotid stenosis associated with a 2-fold increased risk of ipsilateral stroke or cardiovascular death after multifactorial adjustment *(160)*. The absolute risk of an adverse cardiovascular event during the study period was 34% and 17% in those with MMP-9 above and below the median, respectively. In a prospective study of patients with documented coronary artery disease, those who experienced a fatal cardiovascular event during the 4.1-year follow-up period had significantly higher baseline plasma MMP-9 concentrations than those who did not *(161)*. Whether this association provides independent prognostic information compared with other inflammatory markers needs additional assessment.

Conclusion

Contemplation of the clinical use of biomarkers in the context of atherosclerotic cardiovascular disease requires considerable care. Evaluation of the utility of a biomarker requires a clear understanding of the question being asked. Is the task to risk-stratify apparently well or diseased populations? Should the biomarker be measured serially as a target of therapy? Should the biomarker be used as a guide for therapy in addition to the traditional accepted risk factors? Each of these 3 questions requires different types of clinical validation. Many such studies are currently under way.

The revolution in the understanding of the pathophysiology of atherosclerosis has focused attention on inflammation and provided new insight into mechanism of disease. The clinical application of the concept that inflammation participates in atherosclerosis has stimulated the adoption of biomarkers of inflammation in risk prediction and other applications, as noted above. The example of inflammation in atherosclerosis illustrates rapid translation of basic science understanding to the clinic. Further studies, both in progress and on the horizon, will help evaluate the role of novel and emerging biomarkers in the clinical management of atherosclerosis and targeting of therapies.

Although the circulating concentrations of several inflammatory mediators correlate with increased cardiovascular risk, few are ready for clinical practice. CRP attracts particular attention and has stood the test of time, although not all experts agree on its utility. As a downstream biomarker, CRP provides functional integration of overall upstream cytokine activation. Adiponectin, soluble CD40 ligand, IL-18 and MMP-9 are additional biomarkers that have emerged in the search for predictors of a primary adverse cardiovascular event based on clinical data supported by broad experimental evidence. In addition, CRP, sCD40L, and adiponectin may serve as targets for pharmacologic therapy. With the exception of CRP, however, none of the established and emerging novel biomarkers for cardiovascular risk have demonstrated additive value to the Framingham risk score, and few have available commercial assays that achieve adequate levels of standardization and accuracy for clinical use.

Grant/funding Support: This work was supported by a grant from the Fondation Leducq, Paris, France (to Dr. Libby).
Financial Disclosures: Dr. Libby is listed as coinventor on patents held by the Brigham and Women's Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease.

References

1. Libby P, Theroux P. Pathophysiology of coronary artery disease. Circulation 2005;111:3481–8.
2. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol 2006;6:508–19.
3. Libby P, Ridker PM. Novel inflammatory markers of coronary risk: theory versus practice. Circulation 1999;100:1148–50.
4. Libby P, Ridker PM. Inflammation and atherosclerosis: role of C-reactive protein in risk assessment. Am J Med 2004;116 Suppl 6A:9S–16S.
5. Libby P. Inflammation and cardiovascular disease mechanisms. Am J Clin Nutr 2006;83: 456S–460S.
6. Tabas I. Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. J Clin Invest 2002;110:905–11.
7. Castelli WP. Lipids, risk factors and ischaemic heart disease. Atherosclerosis 1996;124 Suppl: S1–9.
8. Steinberg D. Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy, part I. J Lipid Res 2004;45:1583–93.
9. Steinberg D. Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy: part II: the early evidence linking hypercholesterolemia to coronary disease in humans. J Lipid Res 2005; 46:179–90.
10. Steinberg D. Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy, part III: mechanistically defining the role of hyperlipidemia. J Lipid Res 2005;46:2037–51.
11. Steinberg D. The pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy, part IV: the 1984 coronary primary prevention trial ends it—almost. J Lipid Res 2006;47:1–14.
12. Steinberg D. Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy, part V: the discovery of the statins and the end of the controversy. J Lipid Res 2006;47:1339–51.
13. Libby P, Aikawa M, Schonbeck U. Cholesterol and atherosclerosis. Biochim Biophys Acta 2000; 1529:299–309.
14. Glass CK, Witztum JL. Atherosclerosis: the road ahead. Cell 2001;104:503–16.
15. Libby P, Aikawa M. Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. Nat Med 2002;8:1257–62.
16. Skalen K, Gustafsson M, Rydberg EK, Hulten LM, Wiklund O, Innerarity TL, et al. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. Nature (Lond) 2002;417:750–4.
17. Kruth HS. Sequestration of aggregated low-density lipoproteins by macrophages. Curr Opin Lipidol 2002;13:483–8.
18. Williams KJ, Tabas I. The response-to-retention hypothesis of atherogenesis reinforced. Curr Opin Lipidol 1998;9:471–4.
19. Miller YI, Chang MK, Binder CJ, Shaw PX, Witztum JL. Oxidized low density lipoprotein and innate immune receptors. Curr Opin Lipidol 2003;14:437–45.
20. Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science (Wash DC) 1991;251:788–91.
21. Li H, Cybulsky MI, Gimbrone MA Jr, Libby P. An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. Arterioscler Thromb 1993;13:197–204.
22. Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, et al. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. J Clin Invest 2001;107:1255–62.
23. Jongstra-Bilen J, Haidari M, Zhu SN, Chen M, Guha D, Cybulsky MI. Low-grade chronic inflammation in regions of the normal mouse arterial intima predisposed to atherosclerosis. J Exp Med 2006;203:2073–83.
24. Boring L, Gosling J, Cleary M, Charo IF. Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis. Nature (Lond) 1998;394:894–7.
25. Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P, et al. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol Cell 1998;2:275–81.
26. Amorino GP, Hoover RL. Interactions of monocytic cells with human endothelial cells stimulate monocytic metalloproteinase production. Am J Pathol 1998;152:199–207.
27. Rajavashisth TB, Andalibi A, Territo MC, Berliner JA, Navab M, Fogelman AM, et al. Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. Nature (Lond) 1990;344:254–7.
28. Clinton SK, Underwood R, Hayes L, Sherman ML, Kufe DW, Libby P. Macrophage colony-stimulating factor gene expression in vascular cells and in experimental and human atherosclerosis. Am J Pathol 1992;140:301–16.
29. Swirski FK, Libby P, Aikawa E, Alcaide P, Luscinskas FW, Weissleder R, et al. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. J Clin Invest 2007;117:195–205.
30. Tacke F, Alvarez D, Kaplan TJ, Jakubzick C, Spanbroek R, Llodra J, et al. Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. J Clin Invest 2007;117:185–94.
31. Mach F, Sauty A, Iarossi AS, Sukhova GK, Neote K, Libby P, et al. Differential expression of three T lymphocyte-activating CXC chemokines by human atheroma-associated cells. J Clin Invest 1999;104:1041–50.
32. Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci U S A 1995;92:3893–7.
33. Robertson AK, Hansson GK. T cells in atherogenesis: for better or for worse? Arterioscler Thromb Vasc Biol 2006;26:2421–32.
34. Okamoto Y, Kihara S, Funahashi T, Matsuzawa Y, Libby P. Adiponectin: a key adipocytokine in metabolic syndrome. Clin Sci (Lond) 2006;110: 267–78.
35. Matsuzawa Y. Therapy insight: adipocytokines in metabolic syndrome and related cardiovascular disease. Nat Clin Pract Cardiovasc Med 2006;3:35–42.
36. Lara-Castro C, Fu Y, Chung BH, Garvey WT. Adiponectin and the metabolic syndrome: mechanisms mediating risk for metabolic and cardiovascular disease. Curr Opin Lipidol 2007;18: 263–70.
37. Fantuzzi G, Mazzone T. Adipose tissue and atherosclerosis: exploring the connection. Arterioscler Thromb Vasc Biol 2007;27:996–1003.
38. Raines EW, Ferri N. Thematic review series: the immune system and atherogenesis, cytokines affecting endothelial and smooth muscle cells in vascular disease. J Lipid Res 2005;46:1081–92.
39. Mason DP, Kenagy RD, Hasenstab D, Bowen-Pope DF, Seifert RA, Coats S, et al. Matrix metalloproteinase-9 overexpression enhances vascular smooth muscle cell migration and alters remodeling in the injured rat carotid artery. Circ Res 1999;85:1179–85.
40. Gerdes N, Sukhova GK, Libby P, Reynolds RS, Young JL, Schonbeck U. Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for atherogenesis. J Exp Med 2002;195:245–57.
41. Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, et al. Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature (Lond) 1995;378:88–91.
42. Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. Circulation 1999;99: 1726–32.
43. Moulton KS, Vakili K, Zurakowski D, Soliman M, Butterfield C, Sylvin E, et al. Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. Proc Natl Acad Sci U S A 2003;100: 4736–41.
44. Croce K, Libby P. Intertwining of thrombosis and inflammation in atherosclerosis. Curr Opin Hematol 2007;14:55–61.
45. Libby P, Simon DI. Inflammation and thrombosis: the clot thickens. Circulation 2001; 103:1718–20.
46. Mach F, Schonbeck U, Sukhova GK, Bourcier T, Bonnefoy JY, Pober JS, et al. Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis. Proc Natl Acad Sci U S A 1997;94:1931–6.
47. Mach F, Schonbeck U, Bonnefoy JY, Pober JS, Libby P. Activation of monocyte/macrophage functions related to acute atheroma complication by ligation of CD40: induction of collagenase, stromelysin, and tissue factor. Circulation 1997;96:396–9.
48. Schonbeck U, Mach F, Sukhova GK, Murphy C, Bonnefoy JY, Fabunmi RP, et al. Regulation of

matrix metalloproteinase expression in human vascular smooth muscle cells by T lymphocytes: a role for CD40 signaling in plaque rupture? Circ Res 1997;81:448–54.
49. Schonbeck U, Mach F, Sukhova GK, Atkinson E, Levesque E, Herman M, et al. Expression of stromelysin-3 in atherosclerotic lesions: regulation via CD40-CD40 ligand signaling in vitro and in vivo. J Exp Med 1999;189:843–53.
50. Bavendiek U, Libby P, Kilbride M, Reynolds R, Mackman N, Schonbeck U. Induction of tissue factor expression in human endothelial cells by CD40 ligand is mediated via activator protein 1, nuclear factor kappa B, and Egr-1. J Biol Chem 2002;277:25032–9.
51. Schonbeck U, Mach F, Sukhova GK, Herman M, Graber P, Kehry MR, et al. CD40 ligation induces tissue factor expression in human vascular smooth muscle cells. Am J Pathol 2000;156:7–14.
52. Mach F, Schonbeck U, Sukhova GK, Atkinson E, Libby P. Reduction of atherosclerosis in mice by inhibition of CD40 signalling. Nature (Lond) 1998;394:200–3.
53. Schonbeck U, Sukhova GK, Shimizu K, Mach F, Libby P. Inhibition of CD40 signaling limits evolution of established atherosclerosis in mice. Proc Natl Acad Sci U S A 2000;97:7458–63.
54. Hackett D, Davies G, Maseri A. Pre-existing coronary stenoses in patients with first myocardial infarction are not necessarily severe. Eur Heart J 1988;9:1317–23.
55. Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. Circulation 2001; 104:365–72.
56. Amento EP, Ehsani N, Palmer H, Libby P. Cytokines and growth factors positively and negatively regulate interstitial collagen gene expression in human vascular smooth muscle cells. Arterioscler Thromb 1991;11:1223–30.
57. Sukhova GK, Schonbeck U, Rabkin E, Schoen FJ, Poole AR, Billinghurst RC, et al. Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atheromatous plaques. Circulation 1999;99:2503–9.
58. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994;94:2493–503.
59. Gough PJ, Gomez IG, Wille PT, Raines EW. Macrophage expression of active MMP-9 induces acute plaque disruption in apoE-deficient mice. J Clin Invest 2006;116:59–69.
60. Morishige K, Shimokawa H, Matsumoto Y, Eto Y, Uwatoku T, Abe K, et al. Overexpression of matrix metalloproteinase-9 promotes intravascular thrombus formation in porcine coronary arteries in vivo. Cardiovasc Res 2003;57:572–85.
61. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part I. Circulation 2003;108:1664–72.
62. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part II. Circulation 2003;108:1772–8.
63. Vaughan DE. PAI-1 and atherothrombosis. J Thromb Haemost 2005;3:1879–83.
64. Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. N Engl J Med 2000;342:1792–801.
65. Eren M, Painter CA, Atkinson JB, Declerck PJ, Vaughan DE. Age-dependent spontaneous coronary arterial thrombosis in transgenic mice that express a stable form of human plasminogen activator inhibitor-1. Circulation 2002;106:491–6.
66. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352: 1685–95.
67. Luster AD, Alon R, von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. Nat Immunol 2005;6: 1182–90.
68. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med 2006;354:610–21.
69. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol Rev 2006;86:515–81.
70. Maxfield FR, Tabas I. Role of cholesterol and lipid organization in disease. Nature (Lond) 2005;438:612–21.
71. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature (Lond) 2006;444:875–80.
72. Hotamisligil GS. Inflammation and metabolic disorders. Nature (Lond) 2006;444:860–7.
73. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363–9.
74. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004;350:1387–97.
75. Blankenberg S, Yusuf S. The inflammatory hypothesis: any progress in risk stratification and therapeutic targets? Circulation 2006;114:1557–60.
76. Kinlay S, Egido J. Inflammatory biomarkers in stable atherosclerosis. Am J Cardiol 2006;98: 2P–8P.
77. de Lemos JA, Hennekens CH, Ridker PM. Plasma concentration of soluble vascular cell adhesion molecule-1 and subsequent cardiovascular risk. J Am Coll Cardiol 2000;36:423–6.
78. Li H, Cybulsky MI, Gimbrone MA, Jr., Libby P. Inducible expression of vascular cell adhesion molecule-1 by vascular smooth muscle cells in vitro and within rabbit atheroma. Am J Pathol 1993;143:1551–9.
79. Gerszten RE, Luscinskas FW, Ding HT, Dichek DA, Stoolman LM, Gimbrone MA Jr, et al. Adhesion of memory lymphocytes to vascular cell adhesion molecule-1-transduced human vascular endothelial cells under simulated physiological flow conditions in vitro. Circ Res 1996;79: 1205–15.
80. Gerszten RE, Lim YC, Ding HT, Snapp K, Kansas G, Dichek DA, et al. Adhesion of monocytes to vascular cell adhesion molecule-1-transduced human endothelial cells: implications for atherogenesis. Circ Res 1998;82:871–8.
81. Kawakami A, Aikawa M, Alcaide P, Luscinskas FW, Libby P, Sacks FM. Apolipoprotein CIII induces expression of vascular cell adhesion molecule-1 in vascular endothelial cells and increases adhesion of monocytic cells. Circulation 2006;114:681–7.
82. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation 2000;102:2165–8.
83. Zwaka TP, Hombach V, Torzewski J. C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. Circulation 2001;103:1194–7.
84. Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. Circulation 2003;107:398–404.
85. Calabro P, Willerson JT, Yeh ET. Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. Circulation 2003;108:1930–2.
86. Jabs WJ, Theissing E, Nitschke M, Bechtel JF, Duchrow M, Mohamed S, et al. Local generation of C-reactive protein in diseased coronary artery venous bypass grafts and normal vascular tissue. Circulation 2003;108:1428–31.
87. Singh P, Hoffmann M, Wolk R, Shamsuzzaman AS, Somers VK. Leptin induces C-reactive protein expression in vascular endothelial cells. Arterioscler Thromb Vasc Biol 2007;27:e302–7.
88. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285: 2486–97.
89. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002;347:1557–65.
90. Libby P. Inflammation in atherosclerosis. Nature (Lond) 2002;420:868–74.
91. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135–43.
92. 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 2006;47:C19–31.
93. Greenland P, Knoll MD, Stamler J, Neaton JD, Dyer AR, Garside DB, et al. Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. Jama 2003;290:891–7.
94. Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple Risk Factor Intervention Trial. Am J Epidemiol 1996;144:537–47.
95. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973–9.
96. 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;342:836–43.
97. Koenig W, Lowel H, Baumert J, Meisinger C. C-reactive protein modulates risk prediction based on the Framingham Score: implications for future risk assessment: results from a large

cohort study in southern Germany. Circulation 2004;109:1349–53.
98. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, et al. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med 2004;351:2599–610.
99. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 2003;107:391–7.
100. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds risk score. Jama 2007; 297:611–9.
101. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, 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;107:499–511.
102. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. Circulation 1999;100:230–5.
103. Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001;344:1959–65.
104. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, et al. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005;352:20–8.
105. Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, et al. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. N Engl J Med 2005;352:29–38.
106. Ridker PM. Rosuvastatin in the primary prevention of cardiovascular disease among patients with low levels of low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: rationale and design of the JUPITER trial. Circulation 2003;108:2292–7.
107. Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. N Engl J Med 1984;311:501–5.
108. Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease: the Framingham Study. JAMA 1987;258:1183–6.
109. Ma J, Hennekens CH, Ridker PM, Stampfer MJ. A prospective study of fibrinogen and risk of myocardial infarction in the Physicians' Health Study. J Am Coll Cardiol 1999;33:1347–52.
110. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. JAMA 2001;285:2481–5.
111. Mora S, Rifai N, Buring JE, Ridker PM. Additive value of immunoassay-measured fibrinogen and high-sensitivity C-reactive protein levels for predicting incident cardiovascular events. Circulation 2006;114:381–7.
112. Dawson S, Hamsten A, Wiman B, Henney A, Humphries S. Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. Arterioscler Thromb 1991;11:183–90.
113. Festa A, D'Agostino R Jr, Rich SS, Jenny NS, Tracy RP, Haffner SM. Promoter (4G/5G) plasminogen activator inhibitor-1 genotype and plasminogen activator inhibitor-1 levels in blacks, Hispanics, and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. Circulation 2003;107:2422–7.
114. Juhan-Vague I, Alessi MC. PAI-1, obesity, insulin resistance and risk of cardiovascular events. Thromb Haemost 1997;78:656–60.
115. Brown NJ, Agirbasli MA, Williams GH, Litchfield WR, Vaughan DE. Effect of activation and inhibition of the renin-angiotensin system on plasma PAI-1. Hypertension 1998;32:965–71.
116. Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation 1998;98:2241–7.
117. Brown NJ, Abbas A, Byrne D, Schoenhard JA, Vaughan DE. Comparative effects of estrogen and angiotensin-converting enzyme inhibition on plasminogen activator inhibitor-1 in healthy postmenopausal women. Circulation 2002;105: 304–9.
118. Vaughan DE, Rouleau JL, Ridker PM, Arnold JM, Menapace FJ, Pfeffer MA. Effects of ramipril on plasma fibrinolytic balance in patients with acute anterior myocardial infarction. HEART Study Investigators. Circulation 1997;96:442–7.
119. Weber M, Rabenau B, Stanisch M, Elsaesser A, Mitrovic V, Heeschen C, et al. Influence of sample type and storage conditions on soluble CD40 ligand assessment. Clin Chem 2006;52:888–91.
120. Varo N, Nuzzo R, Natal C, Libby P, Schonbeck U. Influence of pre-analytical and analytical factors on soluble CD40L measurements. Clin Sci (Lond) 2006;111:341–7.
121. Lee WL, Lee WJ, Chen YT, Liu TJ, Liang KW, Ting CT, et al. The presence of metabolic syndrome is independently associated with elevated serum CD40 ligand and disease severity in patients with symptomatic coronary artery disease. Metabolism 2006;55:1029–34.
122. de Lemos JA, Zirlik A, Schonbeck U, Varo N, Murphy SA, Khera A, et al. Associations between soluble CD40 ligand, atherosclerosis risk factors, and subclinical atherosclerosis: results from the Dallas Heart Study. Arterioscler Thromb Vasc Biol 2005;25:2192–6.
123. Tayebjee MH, Lip GY, Tan KT, Patel JV, Hughes EA, MacFadyen RJ. Plasma matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-2, and CD40 ligand levels in patients with stable coronary artery disease. Am J Cardiol 2005;96:339–45.
124. Blake GJ, Ostfeld RJ, Yucel EK, Varo N, Schonbeck U, Blake MA, et al. Soluble CD40 ligand levels indicate lipid accumulation in carotid atheroma: an in vivo study with high-resolution MRI. Arterioscler Thromb Vasc Biol 2003;23: e11–4.
125. Schonbeck U, Varo N, Libby P, Buring J, Ridker PM. Soluble CD40L and cardiovascular risk in women. Circulation 2001;104:2266–8.
126. Novo S, Basili S, Tantillo R, Falco A, Davi V, Novo G, et al. Soluble CD40L and cardiovascular risk in asymptomatic low-grade carotid stenosis. Stroke 2005;36:673–5.
127. Hocher B, Liefeldt L, Quaschning T, Kalk P, Ziebig R, Godes M, et al. Soluble CD154 is a unique predictor of nonfatal and fatal atherothrombotic events in patients who have end-stage renal disease and are on hemodialysis. J Am Soc Nephrol 2007;18:1323–30.
128. Heeschen C, Dimmeler S, Hamm CW, van den Brand MJ, Boersma E, Zeiher AM, et al. Soluble CD40 ligand in acute coronary syndromes. N Engl J Med 2003;348:1104–11.
129. Kinlay S, Schwartz GG, Olsson AG, Rifai N, Sasiela WJ, Szarek M, et al. Effect of atorvastatin on risk of recurrent cardiovascular events after an acute coronary syndrome associated with high soluble CD40 ligand in the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) Study. Circulation 2004; 110:386–91.
130. Healy AM, Pickard MD, Pradhan AD, Wang Y, Chen Z, Croce K, et al. Platelet expression profiling and clinical validation of myeloid-related protein-14 as a novel determinant of cardiovascular events. Circulation 2006;113:2278–84.
131. Salmenniemi U, Ruotsalainen E, Pihlajamaki J, Vauhkonen I, Kainulainen S, Punnonen K, et al. Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. Circulation 2004;110:3842–8.
132. Patel DA, Srinivasan SR, Xu JH, Chen W, Berenson GS. Adiponectin and its correlates of cardiovascular risk in young adults: the Bogalusa Heart Study. Metabolism 2006;55:1551–7.
133. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. JAMA 2003;289:1799–804.
134. Chow WS, Cheung BM, Tso AW, Xu A, Wat NM, Fong CH, et al. Hypoadiponectinemia as a predictor for the development of hypertension: a 5-year prospective study. Hypertension 2007;49: 1455–61.
135. Iglseder B, Mackevics V, Stadlmayer A, Tasch G, Ladurner G, Paulweber B. Plasma adiponectin levels and sonographic phenotypes of subclinical carotid artery atherosclerosis: data from the SAPHIR Study. Stroke 2005;36:2577–82.
136. Dullaart RP, de Vries R, van Tol A, Sluiter WJ. Lower plasma adiponectin is a marker of increased intima-media thickness associated with type 2 diabetes mellitus and with male gender. Eur J Endocrinol 2007;156:387–94.
137. Rothenbacher D, Brenner H, Marz W, Koenig W. Adiponectin, risk of coronary heart disease and correlations with cardiovascular risk markers. Eur Heart J 2005;26:1640–6.
138. Lu G, Chiem A, Anuurad E, Havel PJ, Pearson TA, Ormsby B, et al. Adiponectin levels are associated with coronary artery disease across Caucasian and African-American ethnicity.

Transl Res 2007;149:317–23.
139. Otsuka F, Sugiyama S, Kojima S, Maruyoshi H, Funahashi T, Matsui K, et al. Plasma adiponectin levels are associated with coronary lesion complexity in men with coronary artery disease. J Am Coll Cardiol 2006;48:1155–62.
140. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004;291:1730–7.
141. Koenig W, Khuseyinova N, Baumert J, Meisinger C, Lowel H. Serum concentrations of adiponectin and risk of type 2 diabetes mellitus and coronary heart disease in apparently healthy middle-aged men: results from the 18-year follow-up of a large cohort from southern Germany. J Am Coll Cardiol 2006;48:1369–77.
142. Lim S, Koo BK, Cho SW, Kihara S, Funahashi T, Cho YM, et al. Association of adiponectin and resistin with cardiovascular events in Korean patients with type 2 diabetes: the Korean atherosclerosis study (KAS) A 42-month prospective study. Atherosclerosis 2006.
143. Zoccali C, Mallamaci F, Tripepi G, Benedetto FA, Cutrupi S, Parlongo S, et al. Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. J Am Soc Nephrol 2002;13:134–41.
144. Laughlin GA, Barrett-Connor E, May S, Langenberg C. Association of adiponectin with coronary heart disease and mortality: the Rancho Bernardo study. Am J Epidemiol 2007;165:164–74.
145. Wannamethee SG, Whincup PH, Lennon L, Sattar N. Circulating adiponectin levels and mortality in elderly men with and without cardiovascular disease and heart failure. Arch Intern Med 2007;167:1510–7.
146. Hiuge A, Tenenbaum A, Maeda N, Benderly M, Kumada M, Fisman EZ, et al. Effects of peroxisome proliferator-activated receptor ligands, bezafibrate and fenofibrate, on adiponectin level. Arterioscler Thromb Vasc Biol 2007;27:635–41.
147. Samaha FF, Szapary PO, Iqbal N, Williams MM, Bloedon LT, Kochar A, et al. Effects of rosiglitazone on lipids, adipokines, and inflammatory markers in nondiabetic patients with low high-density lipoprotein cholesterol and metabolic syndrome. Arterioscler Thromb Vasc Biol 2006;26:624–30.
148. Forst T, Pfutzner A, Lubben G, Weber M, Marx N, Karagiannis E, et al. Effect of simvastatin and/or pioglitazone on insulin resistance, insulin secretion, adiponectin, and proinsulin levels in nondiabetic patients at cardiovascular risk–the PIOSTAT Study. Metabolism 2007;56:491–6.
149. Skurk T, Kolb H, Muller-Scholze S, Rohrig K, Hauner H, Herder C. The proatherogenic cytokine interleukin-18 is secreted by human adipocytes. Eur J Endocrinol 2005;152:863–8.
150. Thorand B, Kolb H, Baumert J, Koenig W, Chambless L, Meisinger C, et al. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg Study, 1984–2002. Diabetes 2005;54:2932–8.
151. Hung J, McQuillan BM, Chapman CM, Thompson PL, Beilby JP. Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance. Arterioscler Thromb Vasc Biol 2005;25:1268–73.
152. Chapman CM, McQuillan BM, Beilby JP, Thompson PL, Hung J. Interleukin-18 levels are not associated with subclinical carotid atherosclerosis in a community population: the Perth Carotid Ultrasound Disease Assessment Study (CUDAS). Atherosclerosis 2006;189:414–9.
153. Zirlik A, Abdullah SM, Gerdes N, Macfarlane L, Schonbeck U, Khera A, et al. Interleukin-18, the metabolic syndrome, and subclinical atherosclerosis: results from the Dallas Heart Study. Arterioscler Thromb Vasc Biol 2007.
154. Blankenberg S, Tiret L, Bickel C, Peetz D, Cambien F, Meyer J, et al. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. Circulation 2002;106:24–30.
155. Blankenberg S, Luc G, Ducimetiere P, Arveiler D, Ferrieres J, Amouyel P, et al. Interleukin-18 and the risk of coronary heart disease in European men: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). Circulation 2003;108:2453–9.
156. Yasmin, McEniery CM, Wallace S, Dakham Z, Pulsalkar P, Maki-Petaja K, et al. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. Arterioscler Thromb Vasc Biol 2005;25:372.
157. Noji Y, Kajinami K, Kawashiri MA, Todo Y, Horita T, Nohara A, et al. Circulating matrix metalloproteinases and their inhibitors in premature coronary atherosclerosis. Clin Chem Lab Med 2001;39:380–4.
158. Kai H, Ikeda H, Yasukawa H, Kai M, Seki Y, Kuwahara F, et al. Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes. J Am Coll Cardiol 1998;32:368–72.
159. Inokubo Y, Hanada H, Ishizaka H, Fukushi T, Kamada T, Okumura K. Plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 are increased in the coronary circulation in patients with acute coronary syndrome. Am Heart J 2001;141:211–7.
160. Eldrup N, Gronholdt ML, Sillesen H, Nordestgaard BG. Elevated matrix metalloproteinase-9 associated with stroke or cardiovascular death in patients with carotid stenosis. Circulation 2006;114:1847–54.
161. Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G, et al. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation 2003;107:1579–85.
162. Libby P, Ridker PM. Inflammation and atherothrombosis: from population biology and bench research to clinical practice. J Am Coll Cardiol 2006;48:A33–46.