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C-Reactive Protein, Inflammation, & Cardiovascular Disease

Part 1: How Inflammation Affects Atherosclerosis, Thrombosis, & Cardiovascular Risk

Cardiovascular disease accounts for over 725,000 deaths each year in the USA; this represents over 30% of all mortalities and earns it the notorious distinction of claiming more lives than the next two most frequent causes of death combined: cancer and cerebrovascular disease.[1] The worldwide statistics for cardiovascular disease are equally devastating, taking an estimated 14 million lives annually, approximately 20% of all deaths.[2] Ischemic heart disease, which accounts for over 60% of all cardiovascular disease in the USA,[1] is caused primarily by atherosclerosis. Most of these ischemic coronary syndromes — myocardial infarction, unstable angina pectoris, sudden cardiac death, etc. — result from thromboses that develop on or near ruptured atherosclerotic plaques.[3,4,5]

Atherosclerotic plaque composition

Without plaque rupture and resultant occlusive thrombosis, atherosclerosis is often benign and asymptomatic. Morphologic studies of atherosclerosis suggest that the physiologic composition of a plaque, with respect to its susceptibility to rupture, is more significant than the degree of stenosis, or narrowing, of the affected artery in the induction of an acute coronary event.[6] Consistent with this, it has been demonstrated that the degree of stenosis in patients with unstable angina pectoris (UA) is not significantly different from that in patients with stable angina pectoris (SA).[7]

Atherosclerotic plaques consist of two main components: a soft, lipid-rich core and a hard, collagen-rich cap. Generally, coronary plaques are made up of less than 20% lipid-rich core and at least 70% collagen-rich cap.[8,9] Studies have demonstrated that morphologic changes in either the core, the cap, or both can contribute significantly to plaque rupture.

The plaques most vulnerable to rupture tend to have a larger lipid-rich core than stable plaques, comprising 30% to over 40% of the plaque.[10,11] Lipids and other cellular products released from dead, lipid-filled macrophages can increase the size of the core, making the plaque more susceptible to rupture.[12]

The fibrous cap of a plaque is primarily composed of collagen-dense, extracellular matrix. The collagen is secreted by smooth muscle cells in the intima of the affected artery and, once incorporated into the cap, adds rigidity and structural integrity to the entire plaque. In general, plaques will rupture at the area of the cap where the collagen has grown thinnest, usually the shoulder regions.[13,14] Loss or impaired function of these smooth muscle cells can result in thinning of the cap, as ruptured plaques have been shown to contain fewer smooth muscle cells than intact, stable plaques.[11,15]

Inflammation and plaque rupture

A local inflammatory response can also contribute significantly to the thinning of an atherosclerotic cap. Several characteristic features of inflammation — infiltration by inflammatory cells such as macrophages, T-cell lymphocytes, and mast cells, as well as the presence of human leukocyte antigen type DR — have been found at sites of plaque rupture and in surrounding tissues, confirming an active inflammatory reaction.[9,16-24] This inflammation, which facilitates both plaque rupture and subsequent thrombosis, involves the activation of several inflammatory cell types:

Macrophages - Activated macrophages can weaken the fibrous cap of a plaque through degradation of the extracellular matrix by phagocytosis and/or by releasing proteolytic enzymes such as tissue-type plasminogen activator and the matrix metalloproteinases (MMPs) collagenase and stromelysin.[25-28] In addition, macrophage foam cells near a plaque may contribute to plaque instability by furnishing lipids that would increase the relative size of the lipid-rich core. Macrophages can also secrete cytokines such as tumor necrosis factor-α (TNF-α) which can further tissue necrosis in the area of the plaque and increase thrombogenicity.[29]

Mast cells - Activated mast cells are also present in atherosclerotic plaques[30] and in the shoulder area of plaques in particular.[22] It has been proposed that mast cells contribute to the degradation of the extracellular matrix by activating MMPs either directly by secreting MMP-activating proteases, or indirectly by secreting TNF-α, which stimulates macrophages to synthesize MMP-9.[31]

T-cell lymphocytes - Activated T-cells have been found both in atherosclerotic plaques[21,32] and at specific sites of plaque rupture.[16] These activated T-cells can cause smooth muscle cell apoptosis, thus reducing collagen synthesis near a plaque.[27] Additional evidence suggests that T-cells may stimulate smooth muscle cells to express MMP-1, MMP-3, and MMP-9, which may contribute to the degradation of the extracellular matrix and weaken the fibrous caps of nearby plaques.[33]

In addition to the effect local inflammation has on plaque instability and rupture in atherosclerosis, many studies have confirmed the presence of a wide-spread, systemic inflammatory response in cardiovascular disease. Activated macrophages and lymphocytes have been identified not only in the atherosclerotic plaques of cardiac patients, but also in sections of the aorta and coronary arteries that show no signs of plaque formation.[34] Similar evidence of systemic inflammation has been found in the entire coronary tree during cardiac surgery[18] and in the aortic-coronary sinus in right coronary artery disease.[35] In addition to the widespread presence of activated inflammatory cells, other indicators of systemic inflammation have been found in cardiovascular disease; these include altered platelet metabolism,[36] neutrophil activation,[37,38] and increased pro-coagulant activity of monocytes in the presence of lymphocytes.[39] This evidence strongly suggests that inflammation is associated with not only atherosclerosis and plaque rupture, but with cardiovascular disease in general.

Another key component of the inflammatory response, in addition to the activation of the cells discussed above, is an increase in the synthesis and secretion of the acute phase proteins. In cardiovascular disease, inflammation — or an acute phase response — begins with an ischemic event that involves either reversible or irreversible myocardial damage.[40,41]

Abbreviations used in this article

CHD	-	Coronary Heart Disease
CRP	-	C-Reactive Protein
HDL-C	-	High-Density Lipoprotein Cholesterol
IL-1 / -6	-	Interleukin-1 / -6
LDL-C	-	Low-Density Lipoprotein Cholesterol
MI	-	Myocardial Infarction
MMP	-	Matrix Metalloproteinase
PAI-1	-	Plasminogen Activator Inhibitor-1
SA	-	Stable Angina Pectoris
TNF-α	-	Tumor Necrosis Factor-α
TPA	-	Tissue-type Plasminogen Activator
UA	-	Unstable Angina Pectoris

Sidebar: Glycosylation of Acute Phase Proteins in Acute Coronary Syndromes

Most of the acute phase proteins are glycoproteins, and evidence indicates that the specific make-up of their sugar side-chains is altered during an acute phase response. In acute inflammatory reactions, such as those triggered by trauma, burn, tissue damage, or significant psychological stress, most of the acute phase glycoproteins reportedly have more of a particular bi-antennary sugar side-chain, which is identifiable by its strong reactivity with concanavalin A. On the other hand, in the chronic inflammation brought about by long-term, low-grade bacterial infections or rheumatic disease, the sugar side-chains change to more branched, tri-antennary or tetra-antennary structures, which do not react with concanavalin A.[125]

Taking advantage of this difference in binding affinity for concanavalin A, Kazmierczak et al.[126] studied the qualitative and quantitative changes of three acute phase proteins — CRP, α_1-acid glycoprotein, and α_1-antichymotrypsin — in UA and MI patients. The glycosylation profiles of α_1-acid glycoprotein, and α_1-antichymotrypsin were found to change markedly toward more bi-antennary structures, and the plasma levels of all three proteins were significantly elevated in both syndromes. This indicated the presence of an acute inflammatory reaction in these patients. Of note, the qualitative changes in the glycosylation patterns were detected earlier than the quantitative changes, suggesting to the authors that different glycosylated variants of certain acute phase proteins may actually have differing functions based on the specific stimuli eliciting the acute phase response.

At the site of tissue damage, the affected vessel constricts, leukocytes infiltrate the damaged cells, and myocytes and monocytes release cytokine mediators of inflammation which initiate a systemic acute phase reaction. These cytokine mediators, mainly interleukin-6 (IL-6), IL-1, TNF-α, and transforming growth factor, stimulate the liver to increase production of the acute phase proteins. Generally, the acute phase proteins are present at low concentrations in the plasma, but may increase by several hundred-fold during an acute inflammatory response. As early as 1981, increases in acute phase proteins have been associated with myocardial infarction (MI).[42]

Atherosclerosis and thrombosis

Evidence suggests that ruptured plaques are responsible for approximately 75% of all fatal thromboses associated with MI.[5,43,44] Plaque rupture, however, does not always result in occlusive thrombosis.[6] There is evidence to indicate that in as many as 9% of healthy, asymptomatic individuals, plaque rupture occurs without a complicating thrombosis;[45] such cases are often clinically silent and therefore go undetected.

The development of a thrombus at an atherosclerotic plaque occurs under either of two conditions: plaque rupture or subtle erosion of the plaque surface. In either case, thrombus formation is reported to involve three elements:[6]

(1) The local presence of thrombogenic substrates - Elements such as tissue factor, which is reportedly present in higher amounts in MI and UA than in SA,[46] and activated platelets greatly influence thrombogenesis.

(2) Arterial flow dynamics - A large degree of arterial stenosis and the presence of an irregular surface on the plaque can induce thrombogenesis.[47,48,49] These conditions increase the blood velocity and shear forces through the affected area, both of which have been demonstrated to induce platelet aggregation.[50,51]

(3) The thrombotic state of the individual - The presence of unusual thrombotic characteristics such as impaired fibrinolytic capacity, anomalous platelet activation, high fibrinogen levels, and various dysfunctional metabolic states can all affect clot formation.[6]

Many other conditions, such as increased thrombotic potential of the endothelium, neutrophil activation, dysfunctional platelet metabolism, plus various external stresses brought about by biomechanical and hemodynamic shear forces, may contribute to plaque stability/instability and thrombogenesis. These will not be discussed here as they were recently reviewed elsewhere.[6,27]

Thrombus formation appears to relate directly with the symptoms associated with acute coronary syndromes. UA is often characterized by the rapid appearance and disappearance of symptomatic ischemic episodes over a period of days or weeks. These episodes either escalate to MI or subside to SA, as determined by the underlying ischemic stimuli.[27] It is suggested that these ischemic stimuli result from intermittent obstructions of arterial flow caused by non-occlusive or transiently occlusive thromboses.[6] These transiently occlusive thromboses are characteristic of UA patients experiencing chest pain at rest and correlate with non-Q-wave infarction, while occlusive thromboses are more often observed in Q-wave infarction.

Identifying individuals at risk of developing occlusive thromboses remains a challenging task, but assessment of the inflammatory status of an individual may prove useful in this regard. Given the evidence presented above, it appears possible that the inflammatory state of an individual may be useful not only in detecting cardiovascular disease but also in identifying individuals with unstable plaques who may be at greater risk of developing occlusive thromboses.

Novel markers of cardiovascular risk

Presently, in the USA the National Cholesterol Education Program recommends stratifying men and women at risk for cardiovascular disease based on the total number of risk factors present in an individual.[52] These traditional risk factors include:

Family history of coronary heart disease
Cigarette smoking
Hypertension
Hypercholesterolemia (high total cholesterol)
Low levels of high-density lipoprotein cholesterol
Age (men > 45 years, women > 55 years)
Diabetes mellitus

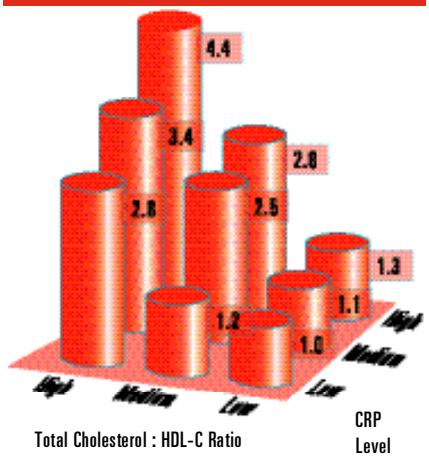
Several new markers have been evaluated in the last several years in an effort to improve the prevention of coronary heart disease (CHD). Among them are lipoprotein(a), homocysteine, fibrinogen and markers of the fibrinolytic system, and markers of inflammation such as CRP. It is important to note that in the evaluation of new markers of cardiovascular risk, three criteria must be met:[53]

(1) Prospective, epidemiologic studies must indicate that the marker is elevated in apparently healthy individuals before the onset of clinical disease.

Note: To accurately assess the utility of a risk factor, it is important that data be accumulated and evaluated prospectively, as opposed to retrospectively as in meta-analyses, to avoid the possibility of the marker being elevated as a result of the acute syndrome being studied.

Figure 1. Relative Risk of Future Myocardial Infarction as Predicted by the Simultaneous Assessment of CRP and Lipid Profile.

(image adapted from reference 120)



(2) Because it is unlikely that total cholesterol and high-density lipoprotein cholesterol (HDL-C) determinations will be replaced soon, new markers must improve the predictive ability currently obtained with the established risk factors of today or must add to the predictive ability of at least one factor.

(3) Standardized assay methods must be available.

In addition to these parameters, it is important whether or not a new marker represents a modifiable risk factor. That is, will therapy intended to control the marker in question result in a reduction in risk? Smoking, hypertension, and hypercholesterolemia represent treatable risk factors and are often the focus of preventive therapy. The goals for treating hypercholesterolemia, as recommended by the National Cholesterol Education Program, are to reach low-density lipoprotein cholesterol (LDL-C) levels of <160 mg/dL in patients without CHD and fewer than two risk factors and <130 mg/dL in patients without CHD, but with two or more risk factors. In addition, it has been suggested that LDL-C levels ≤100 mg/dL should be sought in patients with CHD or other atherosclerotic diseases.[52] With respect to these criteria for

Table 1. New Markers of Cardiovascular Risk and Standard Marker Criteria.[112]

Marker	Additive to Cholesterol Screening	Good Prospective Studies	Assay Standardization
Lipoprotein(a)	Yes/No	Yes/No	No
Homocysteine	Yes/No	Yes/No	Yes/No
TPA/PAI-1	Yes/No	Yes	Yes/No
Fibrinogen	Yes/No	Yes	Yes/No
CRP	Yes	Yes	Yes

markers of cardiovascular risk, the following potential markers have received much attention over the past several years.

Lipoprotein(a) - Lp(a) is a plasma lipoprotein composed of an apolipoprotein B-containing structure, virtually identical to LDL-C, attached to a long, carbohydrate-rich protein, apolipoprotein(a). Lp(a) has been shown to modulate cell surface fibrinolysis, inhibit plasminogen binding to fibrin, and may inhibit tissue-type plasminogen activator (TPA)-catalyzed fibrinolysis.[54,55,56]

High plasma levels of Lp(a) (>30 mg/dL) have shown an association with family history of premature CHD.[57] Prospective studies to date have produced ambiguous results, however, as some studies have shown a positive association between Lp(a) and subsequent cardiovascular disease,[58-63] while several others have not.[64-67] Further investigations have shown that Lp(a) is not an independent marker, but is prognostic only in conjunction with concurrent elevations of LDL-C,[68] and it is unclear whether Lp(a) adds any prognostic information to LDL-C determinations. Given the above, no consensus exists at present regarding the clinical utility of Lp(a) as a marker of cardiovascular risk.

Homocysteine - Homocysteine is a sulfur-containing amino acid that is involved in the metabolism of methionine. A series of studies indicate that plasma levels of homocysteine above 15 µmol/L are associated with an increase in coronary risk.[69,70] It has been suggested that the atherogenic effects of homocysteine may be due to three properties: its effect on endothelial cells, its promotion of pro-coagulant activity, and its adverse effect on platelet adhesion.[71]

Homocysteine increases after MI or stroke,[72,73] indicating its association may be resultant rather than causative. As with Lp(a), prospective studies involving homocysteine have been inconsistent, reporting both positive associations with cardiovascular risk [74-78] and no associations.[79-82] Given these conflicting data, plus the fact that homocysteine assays require much time and effort as they involve high-pressure liquid chromatography, a 1999 report by a subcommittee of the American Heart Association[83] did not recommend adding homocysteine to the screening regimen for cardiovascular risk.

Markers of Fibrinolysis - Abnormalities that result in impaired fibrinolysis may increase the risk for CHD, thrombosis, and stroke.[84,85] The fibrinolytic system is controlled by a balance between plasminogen activators (primarily TPA) and plasminogen inhibitors [primarily plasminogen activator inhibitor-1 (PAI-1)].[86] These factors are synthesized by vascular endothelium, smooth muscle cells, and adipocytes. In general, reduced PAI-1 levels reflect impaired fibrinolytic capacity, resulting in a reduction in plasmin, an accumulation of fibrin, and detrimental enhancement of MMP activity.[87,88] Severe PAI-1 deficiencies in animal models result in spontaneous thrombosis; the same has yet to be demonstrated in humans, but it is reasonable to assume similar adverse results would follow. The over-production of PAI-1 is known to result in hemorrhagic complications in humans.[71] Given these conditions, which may result in abnormal thrombosis or hemorrhaging, it is possible that detection of impaired fibrinolytic capacity may be useful in evaluating future thrombotic risk.

Although positive associations have been reported in prospective studies involving TPA and PAI-1,[89-94] in clinical practice, the assessment of fibrinolytic function has not proven useful. Multivariate analyses indicate that these markers are not independent of other risk factors, and they do not add to the predictive ability of cholesterol screening.[53,95] In addition, fibrinolytic function varies seasonally and with circadian rhythm, which could confound accurate determinations.[96,97] Further complicating evaluation efforts, assessment of fibrinolytic capacity would require standardized phlebotomy conditions, a task difficult to regulate from laboratory to laboratory.

Fibrinogen - Fibrinogen is the precursor of fibrin, the main protein that forms blood clots with platelets and red blood cells. Fibrinogen is also an acute phase protein and thus is intrinsically related both to thrombosis and inflammation.[98,99]

Fibrinogen has been extensively studied and good prospective data exist to link elevated fibrinogen levels with an increased risk of MI, stroke, and general cardiovascular mortality both in men and women.[59,89,100-105] Further investigations have associated fibrinogen with carotid atherosclerosis[106,107] and with future development of CHD.[98] In addition, fibrinogen determinations have added to the predictive value of cholesterol screening[89,103] and have been recommended for use in screening for cardiovascular disease.[98]

Fibrinogen levels increase with age and appear to be related to hormone status as estrogen replacement therapy in women apparently suppresses these age-related increases.[108,109] Fibrinogen is also sensitive to habitual smoking as plasma levels are higher in smokers than in non-smokers. In fact, it has been suggested that approximately half the cardiovascular risk attributable to fibrinogen is linked to smoking,[110,111] implying that the clinical utility of fibrinogen may be limited in non-smokers. The standardization of fibrinogen assays has been problematic as no consensus exists for the preferred method of assay; consequently, both inter- and intra-assay variations are high.[53,112] Although fibrinogen was recommended for use as a screening tool, the drawbacks outlined here suggest that fibrinogen may not be an ideal marker of cardiovascular risk.

Markers of Inflammation / C-Reactive Protein - With inflammation undeniably involved in atherosclerosis, particularly in contributing to plaque instability and rupture, and given that atherosclerosis is the most common cause of acute coronary events, it's no surprise that markers of inflammation are strongly associated with risk of future coronary events.

CRP is perhaps the best studied marker of inflammation, and several prospective, epidemiologic studies have demonstrated a strong association between CRP and cardiovascular risk.[89,113,114,115] Specifically, plasma CRP elevations have consistently indicated a 2- to 3-fold increase in risk of a future coronary event in apparently healthy populations with no history of cardiovascular disease.[116-119] In addition, CRP has demonstrated additive predictive value to cholesterol screening (Figure 1).[118,120] With respect to assay standardization, World Health Organization (WHO) standards exist for CRP and reproducible results are routinely obtained.[121,122,123]

Regarding CRP's status as a modifiable risk factor, although no current treatments specifically target CRP reduction, two preventive therapies appear to reduce plasma CRP: low-dose aspirin and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibition.[113,124] Whether or not preventive therapies designed specifically to reduce plasma CRP levels would also reduce cardiovascular risk remains to be determined.

Given its exemplary performance in each of these three criteria (Table 1), CRP appears at present to be the most promising of these potential markers of cardiovascular risk and will be reviewed in Part 2 of this series.

Summary

Several studies have demonstrated that inflammation is indeed associated with cardiovascular disease. Precisely what stimulates this inflammatory response and whether or not inflammation is a causative factor, however, remain to be determined. At the very least, inflammation contributes to atherosclerotic plaque rupture as inflammatory cells such as macrophages, T-cells, and mast cells tend to concentrate near atherosclerotic plaques and contribute to plaque instability in two ways: (1) dead macrophages release their lipid contents, increasing the size of a plaque's lipid-rich core, and (2) phagocytic or enzymatic degradation of the extracellular matrix weaken the fibrous cap's shoulder regions, pre-disposing them to rupture.

Thus, it appears possible, if not likely, that assessment of the inflammatory state of an individual at risk for an acute coronary event or of a patient already exhibiting cardiac symptoms may aid risk stratification by predicting the likelihood of plaque rupture and subsequent occlusive thrombosis. Although further study is needed, CRP appears at present to be the best candidate marker to serve such a purpose.

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