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# Biomarkers of Plaque Instability

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**Abstract** Atherosclerosis is the proximate cause of arterial thrombosis, leading to acute occlusive cardiovascular syndromes. Thrombosis in atherosclerosis usually results from rupture of the fibrous cap of atherosclerotic plaques with a smaller proportion resulting from superficial endothelial erosion. Ruptured plaques are often associated with intimal and adventitial inflammation, increased size of lipid-rich necrotic core with thinned out collagen-depleted fibrous cap, outward remodeling, increased plaque neovascularity, intraplaque hemorrhage, and microcalcification. By inference, non-ruptured plaques with similar compositional features are considered to be at risk for rupture and hence are labeled vulnerable plaques or high-risk plaques. Identification of vulnerable plaques may help in predicting the risk of acute occlusive syndromes and may also allow targeting for aggressive systemic and possibly local therapies. Plaque rupture is believed to result from extracellular matrix (which comprises the protective fibrous cap) dysregulation due to excessive proteolysis in the context of diminished matrix synthesis. Inflammation is believed to play a key role by providing matrix-degrading metalloproteinases and also by inducing death of matrix-synthesizing smooth muscle cells. Systemic markers of inflammation are thus the most logical forms of potential biomarkers which may predict the presence of vulnerable or high-risk plaques. Several studies have suggested the potential prognostic value of a variety of systemic markers, but regrettably, their overall clinical predictive value is modestly incremental at best, especially for individual subjects compared to groups of patients. Nevertheless, continued investigation of reliable, cost-effective biomarkers that predict the presence of a high-risk plaque and future athero-thrombotic cardiovascular events with greater sensitivity and specificity is warranted.

**Keywords** Atherosclerosis · Inflammation · Plaque · High-risk · Biomarker · Vulnerable · Lipid core

## Introduction

Atherosclerosis is the leading cause of occlusive arterial disease, and atherosclerosis complicated by local thrombosis is the proximate event leading to acute ischemic syndromes such as unstable angina, acute myocardial infarction, ischemic stroke, and many cases of sudden cardiac death [1, 2••]. Thrombosis superimposed on atherosclerotic plaques is most often due to rupture of the fibrous cap of the plaque with exposure of thrombogenic material in the plaque to circulating blood, triggering platelet accumulation, clotting cascade activation, and fibrin deposition [1, 2••]. A smaller proportion of arterial thrombi occurs without plaque rupture and results from superficial endothelial denudation (plaque erosion) coupled with a local or systemic pro-thrombotic state [1, 2••].

The concept of vulnerable or high-risk plaque and the process of plaque rupture are as follows:

Several pathological observations have shown that ruptured plaques with superimposed thrombi tend to have certain compositional features which, by inference, if present in the pre-rupture phase, may identify rupture-prone or vulnerable or high-risk plaques before they rupture [1, 2••]. These compositional features include a large necrotic lipid-rich core, adventitial or outward remodeling, intimal and adventitial inflammation, increased plaque neovascularity and intraplaque hemorrhage, thinning of the fibrous cap with collagen, and smooth muscle cell depletion and microcalcification (Table 1)

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**Table 1** Compositional features of vulnerable or high-risk plaques

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| 1. Large necrotic lipid-rich core |
| 2. Collagen and smooth muscle depleted thin fibrous cap |
| 3. Outward remodeling |
| 4. Inflammation (intimal and adventitial) |
| 5. Neovascularity and intraplaque hemorrhage |
| 6. Microcalcification |

[1, 2••, 3, 4]. The presence of some of these pathological hallmarks has generally been corroborated in living humans by invasive and non-invasive imaging studies such as angioscopy, NIRS imaging, IVUS, CT coronary angiography, OCT, and PET imaging using FDG or sodium fluoride as tracers [5].

Plaque inflammation is believed to play a key role in plaque vulnerability and eventually plaque rupture and thrombosis (Fig. 1) by creating a local milieu that promotes collagen degradation in the fibrous cap and inhibiting collagen synthesis by promoting smooth muscle cell death [1, 2••]. Matrix degrading-enzymes including matrix metalloproteinases (MMP) and other proteases are elaborated by activated inflammatory cells in the plaque, and inflammatory cells also stimulate smooth muscle cell apoptosis through cell-cell contact and humoral mediators of apoptotic cascade; these two processes lead to depletion of collagen in the fibrous cap, its eventual thinning, and rupture either spontaneously or in response to a hemodynamic trigger [1, 2••]. Death of inflammatory cells, specifically lipid-laden foam cells, also contributes to the expansion of the necrotic lipid core, which thus serves as a signature of inflammatory activity in the plaque. Furthermore, intraplaque hemorrhage provides a source of red cell membrane-derived free cholesterol in the plaque [3]. The signatures of inflammatory activity in the plaque thus include thinning of the fibrous cap, large necrotic lipid-rich core

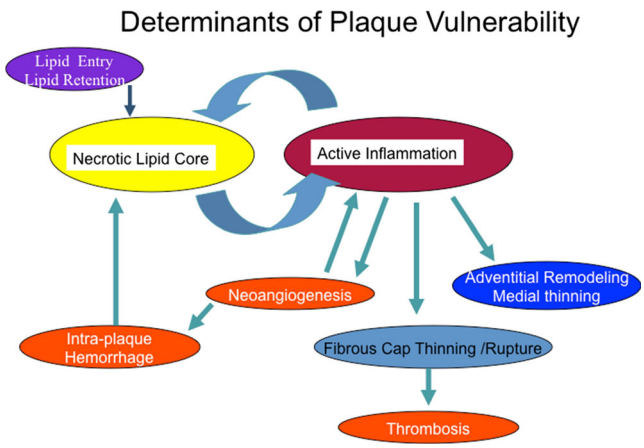


**Fig. 1** The key role of inflammation in atherogenesis and plaque vulnerability providing a rationale for systemic markers of inflammation as markers of plaque vulnerability

impregnated with the procoagulant protein tissue factor derived from inflammatory cells, adventitial remodeling, increased plaque neovascularity, and intraplaque hemorrhage and microcalcification.

## Systemic Biomarkers of Plaque Vulnerability

In view of the central role of inflammation and immune cell activation in determining plaque vulnerability to rupture and thrombosis, systemic markers of inflammation have been evaluated in asymptomatic subjects as well as in acute coronary syndrome patients, to predict the future risk of acute vaso-occlusive cardiovascular events and by inference the presence of high-risk plaques.

## C-Reactive Protein

Of all the biomarkers, C-reactive protein (CRP), a member of the pentraxin family, is the most well-studied biomarker of inflammation that has been evaluated quite extensively for its ability to predict the risk of acute cardiovascular events in various populations of patients including apparently healthy subjects, stable angina patients, and patients with acute coronary syndromes [6]. CRP is predominantly synthesized by hepatocytes under the influence of IL-6, and to a lesser extent, it is also synthesized by plaque-associated smooth muscle cells and macrophage [7–9]. CRP measurement assays have shown analytical stability, reproducibility, and high sensitivity. Some studies have suggested that CRP may also be a culprit in inducing vascular inflammation and atherosclerosis [10], but this has been refuted by other studies which have failed to confirm a causal role for CRP in atherosclerosis and inflammation [11, 12, 13•]. A number of studies have shown that the odds ratios of acute cardiovascular events associated with elevated levels (usually the highest tertile compared to lower tertiles) are somewhere between 1.1 and 4.1, with most under 2.0. A recent meta-analysis involving 160,309 subjects without known cardiovascular disease confirmed the association of CRP levels with several known risk factors and inflammatory markers [14]. In this study, the CRP levels were also related to the risk of future ischemic and non-vascular mortality with odds ratios ranging from 1.44 to 1.71; these odds ratios were further reduced to 1.32 to 1.34 when fibrinogen levels were taken into account [14]. Another analysis of 52 prospective studies was recently performed involving 246,669 participants without a history of cardiovascular disease in whom the value of adding CRP or fibrinogen levels to conventional risk factors for the prediction of cardiovascular risk was ascertained using measures of discrimination and reclassification during follow-up modeling the clinical implications of initiation of statin therapy after the assessment of

CRP or fibrinogen [15•]. This analysis showed that under current treatment guidelines, assessment of the CRP or fibrinogen level in people at intermediate risk for a cardiovascular event could help prevent one additional event over a period of 10 years for every 400 to 500 people screened [15•].

### Interleukin-6

IL-6 is a multifunctional cytokine with pro-inflammatory, pro-atherogenic, and pro-thrombotic effects that is elevated in patients with unstable coronary syndromes where elevated levels are associated with worse outcomes and better response to invasive intervention [6, 16–21]. In addition, prospective studies have shown that elevated levels of IL-6 in asymptomatic subjects without known disease are associated with an increased risk of acute cardiovascular events with odds ratios of 1.4 to 2.9 (mostly below 2.5), but the measurement of IL-6 has not become mainstream since it does not seem to offer any more value than CRP measurement, which can be done reliably with a sensitive, inexpensive, and dependable assay.

### Interleukin 18

Experimental studies have shown that IL-18 has pro-atherogenic effects and IL-18 is also expressed in human atherosclerotic plaques in macrophage-rich regions of the plaque [22–25]. IL-18 can induce IFN gamma and polarize T cells towards a Th1 phenotype as well as induce MMP expression, both of these effects may contribute to the pro-atherogenic effects of IL-18 [26–28]. Clinical studies have shown a less than consistent association between Il-18 levels and CHD events in apparently healthy subjects [29–31] as well as in patients with known CHD [32].

### Secretory PLA2

This enzyme may contribute to atherogenesis and inflammation by favoring lipoprotein retention with vascular proteoglycans, inducing platelet activation through the prostanoid pathway activation and facilitating LDL oxidation [33–40]. In turn, inflammatory mediators may themselves induce secretory PLA2 (sPLA2) production. Relatively small clinical studies have shown adverse prognostic implications of elevated circulating levels of sPLA2 in asymptomatic subjects as well as in subjects with clinically overt stable or unstable coronary artery syndromes [33–40].

The potential pro-atherogenic effect of sPLA2 was recently tested in a randomized clinical trial of an sPLA2 inhibitor, varespladib, with negative results [41]. Thus, at this time, the clinical value of measuring sPLA2 levels remains unclear.

### Lipoprotein-Associated PLA2

This calcium independent phospholipase has been implicated in atherogenesis because it can generate two potent inflammatory mediators (LysoPC and oxFA), which in turn can activate macrophages and SMC as well as induce endothelial dysfunction [42–48]. Two thirds of circulating lipoprotein-associated PLA2 (Lp-PLA2) is carried by LDL particles, whereas the remainder is carried by HDL and VLDL particles [42]. Elevated levels of Lp-PLA2 have been shown to be associated with increased cardiovascular risk independent of other covariates with odds ratios ranging from 1.15 to 2.0, but the overall incremental clinical utility of this biomarker remains unclear. Furthermore, two recent large-scale randomized trials failed to show clinical benefit in known stable or unstable CAD patients with the use of darapladib, an inhibitor of Lp-PLA2 [49, 50]. These results cast further doubt about the potential utility of this biomarker.

### Pregnancy-Associated Plasma Protein A

Pregnancy-associated plasma protein A (PAPP-A) is a zinc-binding metalloproteinase that induces activation of insulin-derived growth factor-1 (IGF-1), which in turn induces inflammation and lipid uptake that can contribute to atherogenesis and plaque instability [51–53]. PAPP-A is predominantly expressed in macrophage-rich regions of ruptured and eroded human atherosclerotic plaques, suggesting a potential role in plaque instability [52]. A small number of clinical studies have shown that elevated levels of PAPP-A in patients with stable and unstable CAD are associated with a higher risk of cardiovascular events, but larger studies are needed to fully evaluate the incremental value of this interesting biomarker [54–59].

### Matrix Metalloproteinases

MMPs are a family of zinc requiring extracellular matrix degrading proteinases, produced by a variety of cells including macrophages, endothelial cells, and smooth muscle cells, capable of inducing breakdown of all components of extracellular matrix, mostly secreted in a pro or zymogen form that requires cleavage into catalytically active enzyme; their activity is inhibited by a family of antagonists called tissue inhibitor of MMP (TIMPS) [60, 61]. MMPs have been implicated in vascular remodeling and possibly plaque cap thinning and rupture in addition to playing a role in other tissues in tissue remodeling, wound healing, and angiogenesis [60]. A family of MMPs has been shown to be expressed in experimental and human atherosclerotic plaques in close association with macrophages [61, 62]. A limited number of human studies have

shown elevated levels of MMPs in patients with advanced CAD and acute coronary syndrome (ACS) patients, and elevated levels in chronic CAD patients have been associated with future risk of cardiovascular events; however, more data are needed to establish the true incremental value of circulating MMP levels [63–66]. Interestingly and contrary to expectation, elevated levels of tissue inhibitor of MMP have also been associated with increased risk of cardiovascular events in patients with CAD [67, 68].

### Myeloperoxidase

Myeloperoxidase is a leucocyte (mostly neutrophil)-derived protease capable of activating MMP and inhibiting TIMP, inducing LDL oxidation through hypochlorous acid generation, inducing oxidation of Apo A-I, and reducing its cholesterol efflux promoting capacity [69–72]. Myeloperoxidase (MPO) is expressed by macrophages in atherosclerotic plaque in close proximity to end products of MPO-induced lipoprotein modification [69]. In apparently healthy subjects, the odds ratio associated with tertile 3 versus tertile 1 for MPO levels was 1.5, whereas odds ratios in ACS have ranged from 1.3 to 4.7 [73]. While elevated levels of MPO in patients with ACS have been observed, the precise incremental clinical utility as a biomarker of risk in patients and apparently healthy subjects remains to be established in large-scale prospective cohorts.

### Oxidized Apo A-1

Over the last decade, it has become abundantly clear that HDL-C levels may not be a reliable biomarker of athero-protective functions of HDL or Apo A-I, especially with HDL-raising therapies, leading to the concept that HDL functionality may be more relevant than HDL-C mass [74]. Recent studies have shown that oxidative modification of Apo A-I by myeloperoxidase may create dysfunctional forms of Apo A-I and HDL that can be detected in atherosclerotic lesions [75•, 76]. Plasma levels of an MPO oxidized from of Apo A-I, called oxTrp72-apo A-I, are elevated in CAD patients and were shown to be associated with an increased cardiovascular risk among 672 subjects, suggesting that such oxidized apo A-I could serve as a potential biomarker for lesional oxidative stress and possibly a vulnerable plaque [75•]. Further studies are clearly indicated to confirm or refute these preliminary observations and define the incremental value of this novel biomarker.

### Placental Growth Factor

This angiogenic protein has been identified in atherosclerotic plaques, and its biological actions are pro-atherogenic as confirmed by gene deletion experiments in mice [77, 78]. Although two studies in acute coronary syndrome patients have suggested an increased risk with elevated levels of placental growth factor (PIGF), its incremental clinical value in predicting outcome in large populations of asymptomatic or symptomatic CAD patients has not been established [79, 80].

### Monocyte Chemo-Attractant Protein-1

Monocyte chemo-attractant protein-1 (MCP-1 or CCL2) is a pro-inflammatory cytokine, which signals through the chemokine receptor 2 (CCR2), is involved in monocyte recruitment in the vessel wall, and is expressed in macrophage-rich regions of plaques [81]. Its pro-atherogenic effects have been confirmed by gene deletion experiments [82]. Although elevated levels of MCP-1 were shown to be associated with a future risk of major adverse events, its independent incremental value as a risk predictor was not confirmed in the MONICA/KORA Augsburg study [83].

### Oxidized LDL

LDL oxidation is thought to occur in the sub-endothelial space, resulting in the generation of a highly pro-inflammatory form of LDL which participates in atherogenesis [84]. Increased circulating levels of oxidized LDL were shown to be a strong predictor of CHD events, independent of other risk factors, in the MONICA/KORA Augsburg study [85]). Further large-scale prospective studies are needed to fully evaluate its incremental prognostic role over and above traditional risk factors.

### Trimethylamine-N-Oxide

It has recently been shown that increased plasma levels of trimethylamine-n-oxide (TMAO) are associated with an increased risk of cardiovascular events independent of conventional risk factors in a 3-year follow-up of 4,007 patients undergoing coronary angiography [86]. Using experimental models, it has been shown that the gut microflora generate trimethylamine (TMA) from dietary sources of choline or phosphatidylcholine (mostly from red meat and dairy products) and that circulating TMA is then converted to TMAO in the liver, TMAO in turn has pro-atherogenic effects since it induces increased macrophage-mediated lipid uptake [87].

Further investigation of this novel and intriguing biomarker would be welcome.

## Summary and Conclusions

Traditional risk assessment algorithms such as the Framingham risk score are useful in identifying individuals at low or high risk for athero-thrombotic cardiovascular events but leave a large group of individuals at intermediate risk where other markers of risk may be of value in reclassifying this cohort into low and high risk. Circulating biomarkers that reflect the critical role of inflammation/oxidative stress in the pathophysiology of athero-thrombosis have been identified and tested for their predictive value in a number of clinical studies. Although statistically significant associations with risk have been identified for a number of such markers, the overall strength of these relationships has been rather weak as reflected by relatively low odds ratios, making them of little incremental value in the clinical setting [88]. This is in sharp contrast to non-invasive imaging assessment of coronary plaque or plaque phenotype by computerized tomography (coronary calcium scan and coronary CT angiography), which provides the best and strongest incremental prognostic information after the Framingham risk assessment with high odds ratios (close to 10) [89]. Future studies of novel biomarkers, perhaps identified by non-biased proteomics/metabolomics studies, must address this issue and demonstrate as good or better predictive power than imaging and or significant incremental predictive power over and above imaging data in order to be clinically useful.

### Compliance with Ethics Guidelines

**Conflict of Interest** Prediman Shah declares that he has no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance


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