Noninvasive Assessment of Atherosclerosis: From Structure to Function

Editor: William Haynes

Previous Brief Reviews in this Series:

Biomarkers of Atherosclerotic Plaque Instability and Rupture

Wolfgang Koenig, Natalie Khuseyinova

Abstract—Basic research over the last two decades has identified a large number of molecules pertinent to the atherosclerotic process, which have clearly improved our understanding of the underlying pathology. It is now well established that inflammation represents a major feature which is present in the vessel wall throughout all stages of the disease until the final pathophysiologic steps, representing plaque destabilization and eventually plaque rupture. Several cells typical for the atherosclerotic plaque, like monocyte-derived macrophages and T-lymphocytes are able to produce and secrete such mediator molecules, like cytokines, chemokines, growth-factors, enzymes, and disintegrins, which lead to activation of endothelial cells, proliferation of smooth muscle cells, lesion progression, and finally to the weakening of a vulnerable plaque by matrix degradation of its fibrous cap. Today, many of these molecules involved can be measured systemically by sensitive assays, and elevated concentrations in the circulation have been shown to be associated with future cardiovascular events. Determination of several of these molecules carries important prognostic information, independent of traditional risk factors, and may turn out to be useful in improving risk stratification. However, for most of these biomarkers the clinical utility has not yet been established. (Arterioscler Thromb Vasc Biol. 2007;27:15-26.)

Key Words: biomarkers ■ atherosclerosis ■ pathophysiology ■ risk prediction

Atherosclerosis is characterized by a complex multifactorial pathophysiology. Inflammation in the vessel wall is now considered to play an essential role in the initiation, progression and the final steps of atherosclerosis, namely plaque destabilization and eventually plaque rupture. Classical pathologic studies show the abundant presence of inflammatory cells, like monocyte-derived macrophages and T-lymphocytes at the site of rupture or superficial erosion preferable in the shoulder area of the plaque cap. These morphological characteristics are preceded by dysfunction of
Activated endothelial cells which produce adhesion molecules that interact with inflammatory cells. The ability of monocyte-derived macrophages to secrete various cytokines, chemokines, growth-factors, and disintegrins, then leads to activation and proliferation of smooth muscle cells, lesion progression, and finally to the weakening of a vulnerable plaque by matrix degradation of its fibrous cap. Yet atherosclerosis and its clinical complications are not only characterized by a local inflammatory response. Recent prospective studies have consistently shown that several molecular proinflammatory biomarkers, markers of plaque destabilization and plaque rupture may be used to predict future cardiovascular (CV) events not only in apparently healthy subjects, but also in patients with acute coronary syndrome (ACS). In particular, measurements of several of these markers carry important prognostic information, independent of traditional risk factors. This review aims at giving an overview on recent biomarker candidates that are related to destabilization and rupture of the atherosclerotic plaque.

C-Reactive Protein
C-reactive protein (CRP) is a member of the pentraxin family and represents the most extensively studied proinflammatory molecule. In healthy individuals, only trace levels of CRP can be detected in the circulation. Under acute conditions, concentrations of CRP increase during the first 6 to 8 hours and can reach peak levels approaching 300 mg/L after approximately 48 hours. CRP is a robust clinical marker because of its analytical stability, shows reproducible results, and high-sensitivity assays with good precision are commercially available. It indicates interleukin, TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein-1; sICAM, soluble intercellular adhesion molecule-1; sVCAM, soluble vascular cell adhesion molecule; oxLDL, oxidized low density lipoprotein; Lp-PLA₂, lipoprotein-associated phospholipase A₂; GPx-1, glutathione peroxidase; MPO, myeloperoxidase; MMPs, matrix metalloproteinases; PIGF, placental growth factor; PAPP-A, pregnancy-associated plasma protein-A; sCD40L, soluble CD40 ligand; CRP, C-reactive protein; sPLA₂, secretory type II phospholipase A₂; SAA, serum amyloid A; WBCC, white blood cell count.

Markers of inflammation and plaque instability: from foam cell to plaque rupture (modified after reference 2) "Biomarkers, which are covered in this review. IL indicates interleukin, TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein-1; sICAM, soluble intercellular adhesion molecule-1; sVCAM, soluble vascular cell adhesion molecule; oxLDL, oxidized low density lipoprotein; Lp-PLA₂, lipoprotein-associated phospholipase A₂; GPx-1, glutathione peroxidase; MPO, myeloperoxidase; MMPs, matrix metalloproteinases; PIGF, placental growth factor; PAPP-A, pregnancy-associated plasma protein-A; sCD40L, soluble CD40 ligand; CRP, C-reactive protein; sPLA₂, secretory type II phospholipase A₂; SAA, serum amyloid A; WBCC, white blood cell count.

Activated endothelial cells which produce adhesion molecules that interact with inflammatory cells. The ability of monocyte-derived macrophages to secrete various cytokines, chemokines, growth-factors, and disintegrins, then leads to activation and proliferation of smooth muscle cells, lesion progression, and finally to the weakening of a vulnerable plaque by matrix degradation of its fibrous cap. Yet atherosclerosis and its clinical complications are not only characterized by a local inflammatory response. Recent prospective studies have consistently shown that several molecular proinflammatory biomarkers, markers of plaque destabilization and plaque rupture may be used to predict future cardiovascular (CV) events not only in apparently healthy subjects, but also in patients with acute coronary syndrome (ACS). In particular, measurements of several of these markers carry important prognostic information, independent of traditional risk factors. This review aims at giving an overview on recent biomarker candidates that are related to destabilization and rupture of the atherosclerotic plaque.
In ROC analyses, CRP did not show an incremental predictive value over traditional risk factors, thereby reinforcing the status of CRP as a strong and independent predictor of future CV risk. Additional data in support of a clinical utility of CRP were generated from the large EPIC-Norfolk study from the UK conducted between 1993 and 2003, which found that CRP was the strongest among the strongest variables predicting risk of coronary heart disease (CHD); and it was the strongest if only fatal cases were analyzed.12 More recently, the large EPIC-Norfolk study from the UK conducted between 1993 and 2003 found that CRP was among the strongest variables predicting risk of coronary heart disease (CHD); and it was the strongest if only fatal cases were analyzed12; and in contrast to the Reykjavik study, the authors found that other risk factors had no incremental value. Additional data in support of a clinical utility of CRP as a predictor variable came from the MONICA Augsburg cohort studies13 and the Cardiovascular Health Study (CHS), an elderly population without a history of vascular disease at baseline.14 Thus, to date, of all biomarkers investigated in CV disease, the most extensive and robust database exists for CRP.
**TABLE 2. Interleukin-6 (IL-6) and Cardiovascular Disease: Overview of Prospective Studies**

<table>
<thead>
<tr>
<th>Author</th>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Outcome Variable</th>
<th>FU</th>
<th>No.</th>
<th>Risk Estimate (RR/OR, 95% CI)*</th>
<th>Reference</th>
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<tr>
<td>Harris et al</td>
<td>Iowa 65+ RHS</td>
<td>case-cohort</td>
<td>Elderly (65+)</td>
<td>CV Mortality</td>
<td>4.6</td>
<td>176/499</td>
<td>2.2 (1.0–4.8)¶</td>
<td>Am J Med 1999; 106:506–512</td>
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<tr>
<td>Ridker et al</td>
<td>PHS</td>
<td>nested case-control</td>
<td>Healthy men</td>
<td>CHD</td>
<td>6</td>
<td>202/202</td>
<td>2.3 (1.1–4.6)†</td>
<td>Circulation 2000; 101:1767–1772</td>
</tr>
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<td>Ridker et al</td>
<td>WHS</td>
<td>nested case-control</td>
<td>Healthy women</td>
<td>OVD</td>
<td>3</td>
<td>122/244</td>
<td>2.2 (1.1–4.3)¶</td>
<td>N Engl J Med 2000; 342:836–843</td>
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<tr>
<td>Volpato et al</td>
<td>WHAS</td>
<td>complete cohort</td>
<td>Elderly (65+)</td>
<td>CV Mortality</td>
<td>3</td>
<td>628</td>
<td>2.52 (2.1–4.5)¶</td>
<td>Circulation 2001;103:947–953</td>
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<tr>
<td>Pradhan et al</td>
<td>WHI</td>
<td>nested case-control</td>
<td>Healthy women</td>
<td>CHD</td>
<td>2.9</td>
<td>304/304</td>
<td>2.1 (1.1–4.0)¶</td>
<td>J Am Med Assoc. 2002; 288:980–987</td>
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<tr>
<td>Cesari et al</td>
<td>Health ABC</td>
<td>complete cohort</td>
<td>Elderly (70+)</td>
<td>CHD/Stroke</td>
<td>3.6</td>
<td>2225</td>
<td>CHD: 1.27 (1.10–1.48)§ Stroke: 1.45 (1.12–1.86)‡</td>
<td>Circulation 2003;108:2317–2322</td>
</tr>
<tr>
<td>Luc et al</td>
<td>PRIME</td>
<td>nested case-control</td>
<td>Health men</td>
<td>CHD</td>
<td>5</td>
<td>317/609</td>
<td>2.16 (1.26–3.72)¶</td>
<td>Arterioscler Thromb Vasc Biol. 2003;23:1255–1261</td>
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<td>Lowe et al</td>
<td>WOSCOPS</td>
<td>nested case-control</td>
<td>Dyslipidemic men</td>
<td>CHD</td>
<td>5</td>
<td>485/934</td>
<td>1.64 (1.11–2.40)§</td>
<td>Arterioscler Thromb Vasc Biol. 2004;24:1529–1534</td>
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</tbody>
</table>

*Multivariable adjustment; †Matched for age and smoking; ¶Increase per 1 standard deviation (SD); (Tertile analysis (T3 vs T1); ‡Quartile analysis (Q4 vs Q1); #Quintile analysis (Q5 vs Q1);

FU indicates Follow-up; CI, Confidence Interval; RR, Relative Risk; OR, Odds Ratio; CHD, Coronary Heart Disease; CVD, Cardiovascular Disease; M, Male; F, Female

**Cytokines**

**IL-6**

IL-6 is a 26-kDa single chain glycoprotein, produced by many cell types including activated monocytes/macrophages and endothelial cells, as well as by adipose tissue. IL-6 is able to stimulate macrophages to secrete MCP-1 and participate in the proliferation of SMCs. In addition, ECs stimulated by IL-6, express intercellular adhesion molecule-1 (ICAM-1). In a murine model of atherosclerosis, injection of excessive amounts of recombinant IL-6 resulted in enhanced fatty lesion development. Furthermore, IL-6 represents the principal procoagulant cytokine, but its most important function is the amplification of the inflammatory cascade through which IL-6 at least in part might exert its direct proatherogenic effects in the arterial wall. Indeed, large amounts of IL-6 have been found in human atherosclerotic plaque, in particular within the shoulder region of stable and unstable plaque, where it colocalized with the angiotensin II type 1 (AT-1) receptor. Upregulation of the AT-1 receptor by IL-6 has led to increased angiotensin II-mediated vasoconstriction, enhanced free oxygen radical production and the development of endothelial dysfunction. In addition, Maier et al have recently demonstrated in patients with ACS that IL-6 levels were markedly higher at the site of coronary plaque rupture than in the systemic circulation.

Various clinical and epidemiological studies have investigated the predictive value of IL-6 plasma concentrations for future CV events. In patients with unstable angina, elevated levels of IL-6 48 hours after admission, were associated with increased in-hospital morbidity and mortality. More importantly, the Fragmin and Fast Revascularization During Instability in Coronary Artery Disease II (FRISC II) study not only confirmed the predictive power of IL-6, but also demonstrated that patients with high IL-6 level might benefit most from an early invasive strategy. In addition, several prospective studies have consistently shown that baseline levels of IL-6 are a potent predictor of future CV end points in apparently healthy asymptomatic subjects from the general population (Table 2).

Thus, IL-6 induces a prothrombotic state and has important direct proatherogenic properties in addition to its role in the amplification of the inflammatory cascade by initiating an acute phase response. Yet, it does not seem suitable for inclusion in the clinical routine, among others because of analytical concerns.

**IL-18**

IL-18, a pleiotropic proinflammatory cytokine, is widely expressed in various cell types. Beyond induction of interferon (IFN)-γ with subsequent promotion of Th1 immune response, IL-18 enhances the expression of matrix metalloproteases (MMPs) and these two abilities of IL-18 characterize it as a crucial and potent mediator of atherosclerotic plaque destabilization and vulnerability. Increased expression of IL-18 in human atherosclerotic plaque has been shown, especially in lesions prone to rupture, where it is localized mainly in plaque macrophages. In animal models, inhibition of IL-18 by IL-18 binding protein reduced atherosclerotic plaque development and progression in apoE-deficient mice and IL-18/apo-E double knockout mice exhibited reduced lesion size, thus further supporting a proatherogenic role of IL-18. In contrast, direct administration of IL-18 enhanced atherosclerosis in an IFN-γ-dependent manner, even in the absence of T-cells and induced/promoted a switch to a vulnerable plaque phenotype by decreasing intimal collagen content and cap-to-core ratio.
Whereas experimental studies on the role of IL-18 in atherogenesis are relatively consistent and promising, the clinical evidence for this biomarker in cross-sectional studies in patients with CHD is controversial. Furthermore, results from one large prospective study, conducted in 1229 patients with angiographically confirmed CHD showed that increased IL-18 levels at baseline were independently associated with future CV death during a 3.9-year FU, but at 5.9 years, IL-18 concentrations were no longer predictive of outcome, thereby questioning its value as a risk marker.

Todate, only two studies assessed the prognostic value of elevated IL-18 for future coronary events in apparently healthy subjects. In the Prospective Epidemiological Study of Myocardial Infarction (PRIME) Study, a cohort from France and Northern Ireland, elevated IL-18 concentrations at baseline were associated with an increased risk for subsequent CHD events after multivariable adjustment. However, such an association was only seen when data from both populations were pooled for analysis. In a large case-cohort study in initially healthy middle-aged men and women from the MONICA/KORA Augsburg populations with a mean FU of 11 years, concentrations of IL-18 were measured in 382 case subjects with incident CHD and in 1980 non-case subjects. In multivariable analyses there was no statistically significant association, neither in men nor in women. This large population-based case-cohort study therefore suggests that IL-18 might only serve as a marker of future CV events in men with manifest CHD and/or in areas of high absolute risk of CHD and thus, further studies are needed to evaluate its true clinical value.

**Oxidized LDL**

The oxidative modification hypothesis of atherogenesis suggests that the most significant event in early lesion formation is lipid oxidation, placing oxidized LDL (oxLDL) in a central role for the development of this disease. OxLDL has a large number of biological actions and consequences, including injuring ECs, expressing adhesion molecules, recruiting leukocytes and retaining them, as well as the formation of foam cells. Furthermore, elevated oxLDL could play a role in the transition from stable to vulnerable, unstable plaque, and this assumption is supported by recent studies showing that oxLDL stimulates matrix metalloproteinase (MMP)-1 and -9 expressions in human vascular EC and in monocyte-derived macrophages. It has also been shown that oxLDL upregulates the expression of MMP-1 and -3 in human coronary ECs, an effect mediated through its endothelial receptor LOX-1. Furthermore, oxLDL triggers the CD40/CD40L signaling pathway, which might also lead to a proinflammatory reaction and induce endothelial injury.

Several cross-sectional studies have examined the involvement of oxidative modification of LDL in subjects with clinical evidence of CHD and demonstrated that oxLDL concentrations were significantly higher in patients with MI than in patients with unstable or stable angina or age-matched controls. Salonen et al were the first to conduct a prospective, population-based, nested case–control study in which the titer of autoantibodies to malondialdehyde-modified LDL and native LDL was associated with accelerated progression of carotid atherosclerosis. More recently, data of a first prospective nested case–control study from two population-based MONICA/KORA Augsburg surveys showed that plasma oxLDL was the strongest predictor of CHD events compared with a conventional lipoprotein profile, and other traditional risk factors for CHD. Further studies are warranted to establish the clinical relevance of oxLDL measurement in various stages of the atherosclerotic process and identify the specific pathophysiological mechanisms by which oxLDL exerts it deleterious effects.

**Glutathion Peroxidase**

Although results of several large antioxidant trials were disappointing, nonetheless, on the basis of experimental and epidemiological studies, it seems justified to assume that oxLDL may indeed play a key role in the generation of inflammatory processes in atherosclerotic lesions and that antioxidative mechanisms still may be important in the pathophysiology of the disease.

Glutathion peroxidase (GPx) is a selenium-containing enzyme with potent antioxidant properties, which uses glutathione to reduce hydrogen peroxide and lipid peroxides to water and lipid alcohols, respectively. To date, 4 isoforms of GPx have been identified, with GPx-1, as an intracellular molecule, being more intensively studied. Experimental studies in GPx-1 knockout mice have demonstrated an increased oxidation of LDL and have developed endothelial dysfunction attributable to deficiency in bioactive nitric oxide as compared with wild-type mice. This enzyme might also inhibit transcription of 5-lipoxygenase as well as leukotriene and prostanoid synthesis in mononuclear cells and macrophages, EC, platelets and leukocytes. In one prospective study, risk of future fatal and non-fatal CV events associated with baseline activity of erythrocyte GPx-1 and superoxide dismutase activity was investigated in 636 patients with angiographically confirmed CHD and was found to be inversely associated with increasing GPx-1 activity. Clearly, such data need replication in further studies, before any sound conclusions can be drawn on its potential value as an additional risk marker.

**Lipoprotein-Associated Phospholipase A<sub>2</sub>**

Lipoprotein-associated phospholipase (Lp-PLA<sub>2</sub>) represents another emerging biomarker for atherosclerotic disease and is presently under intensive investigation. Lp-PLA<sub>2</sub>, a 45.4-kDa protein, is a calcium-independent member of the phospholipase A<sub>2</sub> family. It is produced mainly by monocytes, macrophages, T-lymphocytes, and mast cells and has been found to be upregulated in atherosclerotic lesions, especially in complex plaque, as well as in thin cap coronary lesions prone to rupture. In the bloodstream, two-thirds of the Lp-PLA<sub>2</sub> plasma isoform circulates primarily bound to low-density lipoproteins (LDL), the other third is distributed between HDL and very low-density lipoproteins (VLDL). Lp-PLA<sub>2</sub> may promote oxidation of LDL, and recent investigations have stressed the proatherogenic properties of this enzyme. LDL provides a circulating reservoir, in which Lp-PLA<sub>2</sub> remains inactive until LDL undergoes oxidative modification. After LDL oxidation within the arterial wall, a
TABLE 3. Lp-PLA₂ and Cardiovascular Disease: Overview of Prospective Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Outcome Variable</th>
<th>FU</th>
<th>No.</th>
<th>Risk Estimate (RR/OR, 95% CI)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packard et al†</td>
<td>WOSCOPS nested case-control</td>
<td>Dyslipidemic men</td>
<td>CHD</td>
<td></td>
<td>4.9</td>
<td>590/1160</td>
<td>1.18 (1.05–1.33)†</td>
<td>N Engl J Med. 2000;343:1146–1155</td>
</tr>
<tr>
<td>Blake et al‡</td>
<td>WHS</td>
<td>nested case-control</td>
<td>Healthy women</td>
<td>CHD</td>
<td>3</td>
<td>123/123</td>
<td>1.17 (0.85–3.59)‡</td>
<td>J Am Coll Cardiol. 2001;38:13023106</td>
</tr>
<tr>
<td>Ballantyne et al§</td>
<td>ARIC nested case-control</td>
<td>Healthy M/F</td>
<td>CHD</td>
<td>6–8</td>
<td>608/740</td>
<td>1.15 (0.81–1.63)‡</td>
<td>Circulation: 2004;109:837–842</td>
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</table>

Lp-PLA₂, Type II Secretory Phospholipase A₂

Type II secretory phospholipase A₂ (sPLA₂-II) is another well studied member of the phospholipase A₂ family and is widely expressed in hepatocytes, macrophages, EDs, platelets and vascular SMCs.⁷⁷ SPLA₂ type II is a Ca²⁺-dependent, 14-kDa enzyme which belongs to the group of acute phase reactants. SPLA₂-II production is upregulated in response to proinflammatory compounds such as IL-1β, IL-6, tumor necrosis factor (TNF)-α, INF-γ, and oxLDL.⁷⁷,⁷⁸

Possible atherogenic mechanisms of sPLA₂-II include its effects on lipoproteins which results in the release of various lipid mediators at the site of lipoprotein retention in the arterial wall, that in turn may trigger local inflammatory cellular responses. Furthermore, in arterial tissue, sPLA₂-II may also directly modify LDL particles to become more atherogenic and may increase the affinity of ApoB-100 on LDL to glycosaminoglycans and proteoglycans.⁷⁷,⁷⁹ sPLA₂-II is also implicated in the production of isoprostanes which exhibit strong mitogenic activity and induce platelet aggregation and vasoconstriction. In vivo studies of transgenic mice overexpressing human sPLA₂-II showed an enhanced formation of bioactive oxidized phospholipids, as well as an increased formation of atherosclerotic lesions.⁷⁷,⁸⁰

Furthermore, circulating sPLA₂-II in blood has been demonstrated to predict coronary events in initially healthy subjects and in patients with manifest CHD including ACS. In the EPIC Norfolk study comprising 3314 subjects, elevated levels of sPLA₂ were associated with an increased risk of future CHD events in multivariable analyses.⁸¹ Elevated levels of sPLA₂-II were significant and independent predictors of future cardiovascular events in CHD patients,⁸² in patients with unstable angina,⁸³ and in patients with severe ACS.⁸⁴ Although consistent, all of the above studies in CHD patients were relatively small and results in healthy subjects have to be replicated in other cohorts until the clinical usefulness of sPLA₂-II in the prediction of CHD may be established.
Myeloperoxidase

Myeloperoxidase (MPO), a member of the heme peroxidase superfamily, is a leukocyte-derived enzyme, and is secreted on leukocyte activation and degranulation. There are several pathways through which MPO could exert its deleterious effects. MPO together with other enzymes such as lipoygenase and sPL might initiate lipid oxidation in the subendothelial space of the vessel wall. MPO and its oxidation products have been found to be markedly enriched in human atherosclerotic lesions, compared with control vessels, where they colocalize with macrophages. MPO could be also involved in the development of endothelial dysfunction, because MPO uses the atheroprotective endothelial-derived NO as a substrate. Nonetheless, a most pivotal characteristic of MPO and its end product HOCl remains its ability to activate MMPs and deactivate inhibitors of MMPs, which promote the weakening of the fibrous cap and lead to the destabilized atherosclerotic plaque.

In line with these findings are the results of two prospective studies in patients with ACS. MPO mass concentrations have been measured in the c7E3 Anti-Platelet Therapy in Unstable Refractory angina (CAPTURE) trial in 1090 patients with ACS. Baseline MPO levels predicted an increased risk for adverse CV events, and this effect was even more pronounced in patients without myocardial necrosis (negative for cardiac troponin at baseline). In a large cohort of patients with chest pain, a single measurement of MPO on admission independently predicted acute MI. Thus, MPO might be a promising prognostic marker for CV events, especially in the ACS. However, further studies are needed to replicate these findings and to establish a potential role for MPO as a predictor of incident CHD in initially healthy subjects.

Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) belong to a family of multidomain zinc-dependent endopeptidases that promote degradation of all protein and proteoglycan-core-protein components of the extracellular matrix (ECM). Based on domain organization and substrate specificities, MMPs are grouped into collagenases (MMP1, 8, 13), gelatinases (MMP2 and MMP9), stromelysins (MMP3, 10, and 11), matrilysins (MMP7), metalloelastases (MMP12), and membrane-type (MT)-MMPs. MMPs are widely expressed in monocytes/macrophages, ECs and SMCs, fibroblasts, and neoplastic cells. MMPs are involved in the embryonic development and morphogenesis, wound healing and tissue resorption. On the other hand, MMPs might be implicated in vascular and cardiac remodeling as a result of dysregulated activation of these enzymes. Recently, several lines of evidences have demonstrated that MMPs play an important role in atherosclerosis. Most importantly, MMPs are highly expressed in macrophage-rich areas of the atherosclerotic plaque, especially at the shoulder region of the cap, which might promote weakening of the fibrous cap and subsequent destabilization of atherosclerotic lesions.

Several cross-sectional studies have demonstrated significantly increased concentrations of MMPs in patients with ACS compared with healthy controls or in patients with more advanced CHD. However, to date only one prospective study conducted in 1227 patients with angiographically confirmed CHD, showed that increased concentrations of MMP-9 at baseline were associated with future CV death. Interestingly, high concentrations of the endogenous tissue inhibitors of metalloproteinase-1 (TIMP) were also predictive for future CV death in this study, which has been confirmed by others. Thus, undoubtedly, MMPs play an important role in plaque destabilization, but further studies are needed to prove or disprove their clinical usefulness for risk assessment.

Monocyte Chemotactic Protein-1

Monocyte chemoattractant protein-1 (MCP-1) is the most important chemokine that regulates migration and infiltration of monocytes/macrophages. Its effects are mainly mediated through CC chemokine receptors 2 (CCR2). ECs, monocytes, and/or SMCs express MCP-1 in response to various cytokines, growth factors, oxLDL, and CD40L. and thus MCP-1 expression is increased in atherosclerotic lesions, in particular in macrophage-rich areas. MCP-1 causes chronic vascular inflammation, induces proliferation and migration of SMCs, migration of ECs, neovascularization in plaque, oxidative stress, and thrombosis. Activation of the MCP-1/CCR2 pathway has also been shown to induce expression of MMPs, thus suggesting its involvement in plaque destabilization.

In animal models, the expression of MCP-1 was directly related to the extent of atherosclerosis and macrophage infiltration into the atherosclerotic lesion, and anti-monocyte MCP-1 gene therapy limited the progression and destabilization of established atherosclerosis in apolipoprotein E-knockout mice. Based on these findings, MCP-1 could present an interesting, novel target for intervention to reduce atherosclerotic complications.

Consistent with such experimental data, in the Orifibin trial conducted in 1227 patients with angiographically confirmed CHD, showed that increased concentrations of MMP-9 at baseline were associated with future CV death. Interestingly, high concentrations of the endogenous tissue inhibitors of metalloproteinase-1 (TIMP) were also predictive for future CV death in this study, which has been confirmed by others. Thus, undoubtedly, MMPs play an important role in plaque destabilization, but further studies are needed to prove or disprove their clinical usefulness for risk assessment.

Placental Growth Factor

Placental growth factor (PIGF) represents another important candidate biomarker of plaque instability. PIGF, a member of the cysteine-knot family of growth factors, is a ~50-kDa angiogenic protein, demonstrating an ~40% amino acid sequence similarity to vascular endothelial growth factor (VEGF). PIGF was initially discovered in the placenta, which represents a primary source of its synthesis; further it is expressed in the heart, lungs, goiter, and thyroid tissue and was found to be upregulated within early and advanced atherosclerotic lesions. Besides its physiological functions during pregnancy, PIGF also possesses potent proatherogenic properties such as proliferation and migration of ECs and SMCs, chemo-
tactic recruitment of circulating monocytes and macrophages into atherosclerotic lesions, and upregulation of several cytokines such as, eg, TNF-α. Moreover, PlGF might form a heterodimer with VEGF, thereby enhancing several deleterious effects of this growth factor. Experimental studies using apoE- and PlGF-deficient mice have confirmed a proatherogenic effect of PlGF, demonstrating a reduction of early atherosclerotic plaque development with decreased macrophage content. Furthermore, periadventitial PlGF adenoviral gene delivery to carotid arteries in hypercholesterolemic rabbits led to increased intimal thickening, neointimal macrophage accumulation, and adventitial neovascularization.

Only two clinical studies have investigated the potential role of PlGF as a predictor of adverse outcome in the ACS. Circulating PlGF concentrations were measured in 547 patients of the placebo arm of the CAPTURE trial, as well as in 626 patients presenting to the emergency department with chest pain. Indeed, in these two populations, elevated concentrations of PlGF were significantly associated with an increased risk of adverse events (death or nonfatal MI) at 30 days, and this association was independent of several other biomarkers such as troponin, sCD40L, and CRP. Moreover, when the follow-up period in the CAPTURE study was extended from 1 to 48 months, increased PlGF concentrations remained a potent and independent predictor of the incidence of death or MI. Yet, the present database is still too limited for a recommendation regarding its clinical usefulness as a risk marker.

**Pregnancy-Associated Plasma Protein A (PAPP-A)**

Pregnancy-associated plasma protein A (PAPP-A) is a high-molecular mass, zinc binding metalloproteinase which may be produced by different activated cells in unstable plaques and released into the extracellular matrix. Using specific monoclonal antibodies, PAPP-A was found to be abundantly expressed in both eroded and ruptured coronary and carotid plaques, mainly in monocyte/macrophages present in the cap and shoulder region, but was only minimally expressed in stable plaque. PAPP-A is a specific activator of insulin-like growth factor-1 (IGF-1) and acts by degrading IGF binding proteins-4 and -5, thus allowing active IGF-1 to bind to its receptor and act by degrading IGF binding proteins.

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**Soluble CD40 Ligand (sCD40L)**

CD40 and CD40L (CD 154), both members of the TNF superfamily, are coexpressed by all major cells implicated in atherosclerosis, namely activated T-lymphocytes, vascular ECs, SMCs, and monocytes/macrophages. Both, the receptor and the ligand are functional and CD40/CD40L interactions enhance the expression of various proatherogenic molecules like adhesion molecules, various chemokines (eg, MCP-1), cytokines, growth factors, and MMPs. In addition, CD40L-mediated functions include prothrombotic actions by enhancing the expression of tissue factor and diminishing the expression of thrombomodulin. OxLDL may play a role as an initial trigger of CD40/CD40L expression. The importance of CD40 signaling in atherosclerotic plaque development has been demonstrated using LDL receptor deficient mice. Interrupting CD40 signaling, drastically reduced de novo formation, and progression of established lesions. It also significantly enhanced the lesional content of collagen in mouse atheroma suggesting a change in plaque phenotype. Platelet activation induced by plaque rupture results in increased surface expression of CD40L, which is then cleaved. Circulating soluble (s) CD40L may activate ECs and CD40 expressed in other cells constitutive for the atherosclerotic plaque and induce a proinflammatory cascade in the vessel wall. Human studies indicated that sCD40L is associated with high intraplaque lipid content in patients with carotid atheroma, thus identifying high-risk lesions.

Elevated plasma concentrations of sCD40L have been observed in patients with ACS in the CAPTURE study, which was associated with increased risk for death or non-fatal MI. Patients with elevated sCD40L levels benefited from early antiplatelet therapy with abciximab in this study and also from early statin therapy in the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) study. In another study, the predictive value of sCD40L was shown to be independent of troponin and CRP. Finally, data from the FRISC trial demonstrated that sCD40L levels are modified by a polymorphism in the CD40LG gene, were again related to outcome, and identified a subgroup particularly benefiting from antithrombotic and early invasive treatment. Thus, sCD40L could serve as a
marker of increased thrombotic risk in ACS and may guide aggressive treatment. However, results have to be replicated in further large studies and a number of analytical issues need to be resolved before this biomarker may be used routinely.

Summary and Conclusions

Various molecules involved in the pathogenesis of atherosclerosis predict plaque destabilization and rupture and subsequent clinical complications. They are important research tools and probably useful surrogate markers of atherosclerosis in early clinical “proof of concept” studies.\(^{1,3,4,135}\) But does the measure of a substantial relative risk (usually in the order of 2- to 3-fold) for coronary events qualify these molecules as useful biomarkers for the clinical routine? Probably not, as suggested by the ongoing controversy regarding CRP. For none of the other molecules discussed here the evidence of a predictive value is so robust, and the database is as large as for CRP, yet the incremental value of CRP in clinical decision making has not been ultimately proven.

Recently it has been suggested\(^{136,137}\) that additional criteria need to be applied to a biomarker beyond the independence of its association with an end point, the reliability and accuracy of the test, documented by good sensitivity, specificity, predictive value, and cost-effective issues. Such further test characteristics include likelihood ratios, model calibration, C-statistics, and area under the curve (AUC) in receiver operating characteristic (ROC) analysis. Thus, we still have a long way to go until the most promising candidate molecules have been identified that might help us as clinicians in improving prediction of the deleterious clinical complications of atherosclerosis. To date, none of the biomarkers discussed here has been recommended to the physician for routine clinical use.

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