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
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 chemotactrant 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 cap1 (Figure). 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).2 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.3 CRP is a robust clinical marker because of its analytical stability, shows reproducible results, and high-sensitivity assays with good precision are commercially available.

CRP is synthesized by hepatocytes and its production is under transcriptional control of several cytokines, with interleukin (IL)-6 being a primary stimulus. However, recent evidence has suggested that CRP may be also produced locally in vascular smooth muscle cells (SMCs) and macrophages of atherosclerotic lesions.4–5 A recent post-mortem study further confirmed a potential pathogenic role of CRP in atheromatous plaque vulnerability,6 demonstrating that higher CRP concentrations strongly correlated with increased numbers of thin cap fibroatheromas.

CRP has initially been considered as an innocent bystander in the atherosclerotic process. Recent evidence, however, suggests that CRP may have direct proinflammatory effects, and contribute to the initiation, and progression of atherosclerotic lesions. Functionally, CRP has several effects that may influence progression of vascular disease, including activation and chemotraction of circulating monocytes, mediation of endothelial dysfunction, induction of a prothrombotic state, increase of cytokine release, activation of the complement system, facilitation of extracellular matrix remodeling as well as lipid-related effects.7 However, several of the above mentioned potential proatherogenic properties of CRP should be interpreted with caution, because very recent findings indicated that several direct effects of CRP on the vasculature, observed in in vitro studies, might represent artifacts.8 Animal models, including transgenic mice, have also provided conflicting evidence regarding proatherogenic effects of CRP.7

In contrast to the controversial results from vitro and in vivo studies supporting a causal role for CRP in atherogenesis, epidemiological studies published during the past decade have provided strong evidence for CRP to predict future CV risk in a wide variety of clinical settings, including apparently healthy men and women, patients with stable angina pectoris (AP), or those with ACS, after myocardial infarction (MI), and with the metabolic syndrome.

So far, results from more than 25 different prospective studies have been reported, and the vast majority of these studies clearly demonstrated a significant and independent association between increased concentrations of CRP and future CV events (Table 1). An earlier meta-analysis summarizing the results of 14 prospective long-term studies with a total of 2557 cases and a mean follow up (FU) period of 8 years9 revealed a summary relative risk (RR) for CHD of 1.9 (95% CI, 1.25 to 2.3) for the top versus the bottom tertile (T) of the CRP distribution. More recently, however, the results from the Reykjavik study, raised some uncertainties regarding the predictive power of CRP by showing a more modest increased risk associated with elevated CRP concentrations with an odds ratio (OR) of 1.45 (95% CI, 1.25 to 1.68) for T3 versus T1 after multivariate adjustment.10 A subsequent meta-analysis of 22 population-based studies, including a total of 7068 patients with incident coronary events, showed a similar result.10 However, there are some issues concerning this study, which merit consideration. The Reykjavik study represents a prospective cohort of 18 569 participants, where CRP was measured in approximately 6500 middle-aged men and women without a history of MI at baseline, who were followed for 17.5 years. As compared with previously published studies, the Reykjavik participants had the highest cholesterol levels seen in any
cohort or clinical trial, even higher than in the 4 S study,13 and lower CRP (upper tertile cut-off point of 2.0 mg/L, rather that 3.0 mg/L) as seen in almost all other studies. Thus, underestimation of the true risk associated with elevated CRP is very likely. In addition, the mean FU period of 17 years is extremely long and might also be responsible for the weakening of the association between the risk marker and the disease outcome. A phenomenon that is well known from classical risk factors. Indeed, if we look at the risk estimate at 10 years ("normal" FU time in most prospective studies) the risk estimate was 1.84 (95% CI, 1.49 to 2.28), that is similar to those from other studies, thereby reinforcing the status of CRP as a strong and independent predictor of future CV risk. In ROC analyses, CRP did not show an incremental predictive value once total cholesterol was in the model, but other classical risk factors, like smoking and hypertension did not either. 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 Reyjkjavik 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

### Table 1. C-Reactive Protein and Cardiovascular Disease: Overview of Prospective Studies

<table>
<thead>
<tr>
<th>Author et al</th>
<th>Study Design</th>
<th>Population</th>
<th>Outcome Variable</th>
<th>FU</th>
<th>No.</th>
<th>Risk Estimate (Relative Risk, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuller et al</td>
<td>MRFIT nested case-control</td>
<td>Healthy men</td>
<td>CHD</td>
<td>10</td>
<td>246/491</td>
<td>1.54 (0.96–2.52)§</td>
</tr>
<tr>
<td>Ridker et al</td>
<td>PHS nested case-control</td>
<td>Healthy men</td>
<td>CHD</td>
<td>8</td>
<td>543/543</td>
<td>CHD: 2.9 (2.0–4.8); Stroke: 1.9 (1.3–2.9)§</td>
</tr>
<tr>
<td>Tracy et al</td>
<td>CHS nested case-control</td>
<td>Elderly (65+)</td>
<td>CHD</td>
<td>2.4</td>
<td>146/146</td>
<td>2.67 (1.04–6.6)§</td>
</tr>
<tr>
<td>Tracy et al</td>
<td>RHPP nested case-control</td>
<td>Elderly (65+)</td>
<td>CHD</td>
<td>3</td>
<td>145/146</td>
<td>M: 2.5 (0.8–8.4)§</td>
</tr>
<tr>
<td>Koenig et al</td>
<td>MONICA Augsburg complete cohort</td>
<td>Healthy men</td>
<td>CHD</td>
<td>12</td>
<td>323/258</td>
<td>RR not assessed; P = 0.3346</td>
</tr>
<tr>
<td>Koenig et al</td>
<td>MONICA/KORA complete cohort</td>
<td>Healthy men</td>
<td>CHD</td>
<td>8.2</td>
<td>936</td>
<td>1.50 (1.04–2.17)§</td>
</tr>
<tr>
<td>Harris et al</td>
<td>Iowa 65+ RIS</td>
<td>case-control</td>
<td>Healthy (65+)</td>
<td>CD / Mortality</td>
<td>4.6</td>
<td>176/499</td>
</tr>
<tr>
<td>Jager et al</td>
<td>Hoorn complete cohort</td>
<td>Nondiab/diab M/F</td>
<td>CV Mortality</td>
<td>5</td>
<td>631</td>
<td>1.93 (0.61–6.0)§</td>
</tr>
<tr>
<td>Rost et al</td>
<td>Framingham complete cohort</td>
<td>Healthy M/F</td>
<td>Stroke</td>
<td>12–14</td>
<td>1462</td>
<td>M: 1.6 (0.67–3.3)§</td>
</tr>
<tr>
<td>Piro et al</td>
<td>Quebec CV</td>
<td>complete cohort</td>
<td>Healthy men</td>
<td>5</td>
<td>2037</td>
<td>11.1 (0.7–1.6)§</td>
</tr>
<tr>
<td>Love et al</td>
<td>Speedwell</td>
<td>complete cohort</td>
<td>Healthy men</td>
<td>6.3</td>
<td>2055</td>
<td>1.60 (0.90–2.8)§</td>
</tr>
<tr>
<td>Albert et al</td>
<td>PHS nested case-control</td>
<td>Healthy men</td>
<td>SCID</td>
<td>17</td>
<td>97/192</td>
<td>2.65 (0.79–8.3)§</td>
</tr>
<tr>
<td>Pradhan et al</td>
<td>WHI</td>
<td>nested case-control</td>
<td>Healthy women</td>
<td>2.9</td>
<td>304/304</td>
<td>2.1 (1.0–4.1)§</td>
</tr>
<tr>
<td>Ridker et al</td>
<td>WHS</td>
<td>nested case-control</td>
<td>Healthy women</td>
<td>8</td>
<td>293/293</td>
<td>2.3 (1.6–3.4)§</td>
</tr>
<tr>
<td>Luc et al</td>
<td>PRIME</td>
<td>nested case-control</td>
<td>Healthy men</td>
<td>5</td>
<td>317/609</td>
<td>2.16 (1.36–3.72)§</td>
</tr>
<tr>
<td>Van der Meer et al</td>
<td>Rotterdam</td>
<td>nested case-control</td>
<td>Healthy men</td>
<td>5</td>
<td>157/500</td>
<td>1.20 (0.6–2.2)§</td>
</tr>
<tr>
<td>Koenig et al</td>
<td>Reyjkjavik</td>
<td>nested case-control</td>
<td>Healthy M/F</td>
<td>17.5</td>
<td>2459/969</td>
<td>1.45 (0.25–1.68)§</td>
</tr>
<tr>
<td>Babeynie et al</td>
<td>ARIC</td>
<td>nested case-control</td>
<td>Healthy M/F</td>
<td>6–8</td>
<td>606/740</td>
<td>1.72 (1.24–2.38)§</td>
</tr>
<tr>
<td>Pol et al</td>
<td>NHS</td>
<td>nested case-control</td>
<td>Healthy M/F</td>
<td>8</td>
<td>239/469</td>
<td>1.53 (0.89–2.62)§</td>
</tr>
<tr>
<td>Cushman et al</td>
<td>HPFU</td>
<td>complete cohort</td>
<td>Healthy M/F</td>
<td>6</td>
<td>265/529</td>
<td>M: 1.79 (1.14–2.6)§</td>
</tr>
<tr>
<td>Cushman et al</td>
<td>CHS</td>
<td>complete cohort</td>
<td>Elderly (65+)</td>
<td>10</td>
<td>3971</td>
<td>1.37 (0.96–1.73)§</td>
</tr>
<tr>
<td>Laksarones et al</td>
<td>KHD</td>
<td>complete cohort</td>
<td>Healthy men</td>
<td>14.6</td>
<td>1478</td>
<td>2.94 (0.46–5.9)§</td>
</tr>
<tr>
<td>Wilson et al</td>
<td>PHS</td>
<td>complete cohort</td>
<td>Healthy M/F</td>
<td>8</td>
<td>4446</td>
<td>1.16 (0.92–1.47)§</td>
</tr>
<tr>
<td>Boekhildt et al</td>
<td>EPIC-Nordic</td>
<td>nested case-control</td>
<td>Healthy men</td>
<td>6</td>
<td>1106/2164</td>
<td>1.66 (1.31–2.12)§</td>
</tr>
<tr>
<td>Koenig et al</td>
<td>MONICA/KORA</td>
<td>case-control</td>
<td>Healthy M/F</td>
<td>11</td>
<td>382/1980</td>
<td>M: 1.19 (0.82–2.77)§</td>
</tr>
</tbody>
</table>

*Multivariable adjustment; †Partially adjusted; §Increase per 1 Unit or standard deviation (SD); || vs < median; ¶Tertile analysis (T3 vs T1); #Quartile analysis (Q5 vs Q1); $For CRP > 3 mg/L vs CRP < 3 mg/L; FU indicates Follow-up; CI, Confidence Interval; RR, Relative Risk; OR, Odds Ratio; CHD, Coronary Heart Disease; CVD, Cardiovascular Disease; SCID, Sudden Cardiac Death; M, Male; F, Female; MRFIT, Multiple Risk Factor Intervention Trial; PHS, Physicians’ Health Study; CHS, Cardiovascular Health Study; RHPP, Rural Health Promotion Project; WHS, Women’s Health Study; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; Iowa 65+ RIS, Iowa 65+ Rural Health Study; BRHS, British Regional Heart Study; WOSCOPS, West of Scotland Coronary Prevention Study; WHI, Women’s Health Initiative; PRIME, Prospective Epidemiological Study of Myocardial Infarction; ARIC, Atherosclerosis Risk in Communities; NHS, Nurses’ Health Study; HPFS, Health Professionals Follow-Up Study; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study; FHS, Framingham Heart Study; KORA, Cooperative Gesundheitsforschung in der Region Augsburg.

In ROC analyses, CRP did not show an incremental predictive value once total cholesterol was in the model, but other classical risk factors, like smoking and hypertension did not either. More recently, the large EPIC-Nordic 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 Reyjkjavik 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
**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>Risk Estimate (RR/OR, 95% CI)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<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>
</tr>
<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>
</tr>
<tr>
<td>Ridker et al</td>
<td>WHS</td>
<td>nested case-control</td>
<td>Healthy women</td>
<td>CHD</td>
<td>3</td>
<td>122/244</td>
<td>2.2 (1.1–4.3)</td>
</tr>
<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>629</td>
<td>2.52 (2.1–4.5)</td>
</tr>
<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>
</tr>
<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>
</tr>
<tr>
<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>
</tr>
</tbody>
</table>

*Multivariable adjustment; †Matched for age and smoking; ‡Increase per 1 standard deviation (SD); |T(ertile 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; Iowa 65+; RHS, Iowa 65+ Rural Health Study; PHS, Physicians’ Health Study; WHS, Women’s Health Study; WHAS, Women’s Health and Aging Study; WHI, Women’s Health Initiative; Health ABC, Health, Aging, and Body Composition Study; PRIME, Prospective Epidemiological Study of Myocardial Infarction; WOSCOPS, West of Scotland Coronary Prevention Study; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease/KORA, Kooperative Gesundheitsforschung in der Region Augsburg

**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 macrophores to secrete MCP-1 and participates 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 exerts 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, further supporting a proatherogenic role of IL-18. In contrast, direct administration of IL-18 enhanced atherogenesis 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. \(^{40-42}\) 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,\(^ {43}\) but at 5.9 years, IL-18 concentrations were no longer predictive of outcome,\(^ {44}\) thereby questioning its value as a risk marker.

Today, only two studies assessed the prognostic value of elevated IL-18 for future coronary events in apparently healthy subjects.\(^ {45-46}\) In the Prospective Epidemiological Study of Myocardial Infarction (PRIME) Study,\(^ {45}\) 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,\(^ {46}\) 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.\(^ {47}\) 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 monococyte-derived macrophages.\(^ {48-49}\) 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.\(^ {50}\) Furthermore, oxLDL triggers the CD40/CD40L signaling pathway, which might also lead to a proinflammatory reaction and induce endothelial injury.\(^ {51}\)

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.\(^ {52-54}\) Salonen et al\(^ {55}\) 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\(^ {56}\) 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 its deleterious effects.

### Lipoprotein-Associated Phospholipase A\(_2\)

Lipoprotein-associated phospholipase (Lp-PLA\(_2\)) represents another emerging biomarker for atherosclerotic disease and is presently under intensive investigation. Lp-PLA\(_2\), a 45.4-kDa protein, is a calcium-independent member of the phospholipase A\(_2\) 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.\(^ {64}\) In the bloodstream, two-thirds of the Lp-PLA\(_2\) plasma isoform circulates primarily bound to low-density lipoproteins (LDL), the other third is distributed between HDL and very low-density lipoproteins (VLDL).\(^ {64,65}\) Lp-PLA\(_2\) may promote oxidation of LDL, and recent investigations have stressed the proatherogenic properties of this enzyme.\(^ {66}\) LDL provides a circulating reservoir, in which Lp-PLA\(_2\) remains inactive until LDL undergoes oxidative modification. After LDL oxidation within the arterial wall, a
short acyl group at the sn-2 position of phospholipids becomes susceptible to the hydrolytic action of Lp-PLA₂ that cleaves an oxidized phosphatidylcholine component of the lipoprotein particle generating two potent proinflammatory mediators, namely lysophosphatidylcholine (LysoPC) and oxidized fatty acid (oxFA). LysoPC and proatherogenic mediators, namely lysophosphatidylcholine, exhibit strong mitogenic activity and induce platelet aggregation and vascular SMCs. SPLA₂-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. 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.スPLA₂-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.

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. Elevated levels of spla₂-II were significant and independent predictors of future cardiovascular events in CHD patients, in patients with unstable angina, and in patients with severe ACS. 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 in the prediction of CHD may be established.

### 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</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<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 (0.45–3.59)</td>
<td>J Am Coll Cardiol. 2001;38:13023106</td>
</tr>
<tr>
<td>Ballantyne et al</td>
<td>ARIC</td>
<td>nested case-cohort</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>
</tr>
<tr>
<td>Koenig et al</td>
<td>MONICA</td>
<td>complete cohort</td>
<td>Healthy man</td>
<td>CHD</td>
<td>14</td>
<td>934</td>
<td>1.21 (1.01–1.45)</td>
<td>Circulation. 2004;110:1903–1908</td>
</tr>
<tr>
<td>Oei et al</td>
<td>Rotterdam</td>
<td>nested case-cohort</td>
<td>Elderly (55+)</td>
<td>CHD</td>
<td>10</td>
<td>377/1822</td>
<td>1.76 (0.98–3.25)</td>
<td>Circulation. 2005;111:570–575</td>
</tr>
<tr>
<td>Persson et al</td>
<td>Malmö</td>
<td>complete cohort</td>
<td>Healthy M/F</td>
<td>CVD</td>
<td>9.4</td>
<td>145/4938</td>
<td>1.69 (1.10–3.09)</td>
<td>Circulation. 2005;112:802</td>
</tr>
<tr>
<td>Britakos et al</td>
<td>Mayo</td>
<td>complete cohort</td>
<td>CHD patients</td>
<td>CVD</td>
<td>4</td>
<td>504</td>
<td>1.30 (0.66–2.59)</td>
<td>Arterioscler Thromb Vasc Biol. 1999;19:1597–1603</td>
</tr>
<tr>
<td>Horne et al</td>
<td>IHCS</td>
<td>complete cohort</td>
<td>CHD patients</td>
<td>CHD death</td>
<td>6.7</td>
<td>1493</td>
<td>1.73 (0.84–3.51)</td>
<td>Arterioscler Thromb Vasc Biol. 1999;19:1597–1603</td>
</tr>
<tr>
<td>Persson et al</td>
<td>Malmö</td>
<td>complete cohort</td>
<td>Healthy M/F</td>
<td>Stroke</td>
<td>9.4</td>
<td>112/4938</td>
<td>1.69 (0.90–3.28)</td>
<td>Circulation. 2005;112:802</td>
</tr>
<tr>
<td>Koenig et al</td>
<td>KAROLA</td>
<td>complete cohort</td>
<td>Healthy M/F</td>
<td>CVD</td>
<td>4</td>
<td>1051</td>
<td>2.09 (1.30–3.36)</td>
<td>Arterioscler Thromb Vasc Biol. 2006;26:1586–1593</td>
</tr>
<tr>
<td>Elkind et al</td>
<td>NOMAS</td>
<td>complete cohort</td>
<td>Stroke patients</td>
<td>Stroke</td>
<td>4.0</td>
<td>467</td>
<td>2.08 (1.09–4.18)</td>
<td>Arch Intern Med. 2006;166:2073–2080</td>
</tr>
</tbody>
</table>

*Multivariable adjustment; †Measurement of Lp-PLA₂ mass; ‡ Measurement of Lp-PLA₂ activity; ||Increase per 1 standard deviation (SD); ††Tertile analysis, (T3 vs T1); †‡Urartile analysis, (Q4 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; WOSCOPS, West of Scotland Coronary Prevention Study; WHS, Women’s Health Study; ARIC, Atherosclerosis Risk in Communities; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; KAROLA, Langzeitfolge der kardiologischen Anschlußheilbehandlung; IHCS, Intermountain Heart Collaborative Study; NOMAS, Northern Manhattan Study.
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 lipoxygenase 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 MPO87 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 Chemoattractant Protein-1
Monocyte chemoattractant protein-1 (MCP-1) (CCL2) 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, 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 Orbofiban in Patients with Unstable coronary Syndromes (OPUS)-TIMI 16 trial, elevated levels of MCP-1 were associated with risk of death or MI after 10 months, independent of a variety of CV risk factors, clinical and ECG characteristics, renal function, and markers of necrosis and inflammation. However, although in a large case-cohort study from the MONICA/KORA Augsburg database, elevated levels of MCP-1 preceded CHD events, they were not independent predictors of risk, once traditional risk factors were also considered. Thus, further studies in various populations are needed to potentially establish MCP-1 as a clinically useful biomarker.

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**

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 only was 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 growth factor-1 (IGF-1) and acts by degrading IGF binding in stable plaque. PAPP-A is a specific activator of insulin-like growth factor-1 (IGF-1) and acts by degrading IGF binding in stable plaque. 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 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 cell-surface type 1 IGF receptors. IGF-1 induces cell proliferation, differentiation, migration, inflammatory cell activation, LDL-cholesterol uptake, and release of inflammatory cytokines, thus contributing to plaque progression and destabilization. Whether PAPP-A directly can degrade extracellular matrix remains unclear.

Several studies in patients with ACS, but also with stable CHD, have investigated PAPP-A as a potential marker of risk for clinical complications. In a small study, circulating PAPP-A levels were significantly higher in patients with unstable angina and MI compared with controls. In a larger cohort of 200 patients with troponin negative ACS, PAPP-A levels independently predicted ischemic cardiac events and need for revascularization during 6-month FU. Within the CAPTURE trial, PAPP-A levels indicated increased risk of death and MI in both troponin negative and troponin positive patients. In multivariable analysis, PAPP-A, sCD40L, IL-10, and VEGF were independent predictors of outcome. Sim-ilarly, in patients with STEMI, PAPP-A levels were increased and predicted 12-month risk of death and recurrent non-fatal MI. In addition, PAPP-A and its endogenous inhibitor, the proform of eosinophil major basic protein (proMBP), were related to complex angiographic stenosis morphology in patients with stable CHD, and PAPP-A was prospectively associated with future death and ACS in such patients. Thus, in several studies increased circulating PAPP-A levels have been shown as a mediator of adverse inflammatory events, but it has also been suggested that PAPP-A may be a suppressor rather than a mediator of inflammation and tissue damage. Also, there is recent evidence for the presence of an ACS-related isof orm of PAPP-A, which is not complexed with the proMBP, that should result in the development of more specific assays. Thus, further mechanistic and clinical studies are needed to assess the potential utility of PAPP-A for risk stratification in the ACS.

**Soluble CD40 Ligand**

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 (MIRA CL) 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. 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 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 atherosclerotic plaques. To date, none of the biomarkers discussed here can be recommended to the physician for routine clinical use.

Disclosures

Wolfgang Koenig has received an unrestricted grant from diaDexus, GlaxoSmithKline, Roche, and Abbott.

References


Koenig and Khuseyinova Biomarkers and Plaque Destabilization

23


76. Hurt-Camejo E, Camejo G, Peilot H, Oorni K, Kovanen P. Phospho-
77. Hurt-Camejo E, Camejo G, Peilot H, Oorni K, Kovanen P. Phospho-


Biomarkers of Atherosclerotic Plaque Instability and Rupture
Wolfgang Koenig and Natalie Khuseyinova

Arterioscler Thromb Vasc Biol. 2007;27:15-26; originally published online November 2, 2006;
doi: 10.1161/01.ATV.0000251503.35795.4f
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/27/1/15

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the
Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for
which permission is being requested is located, click Request Permissions in the middle column of the Web
page under Services. Further information about this process is available in the Permissions and Rights
Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online
at:
http://atvb.ahajournals.org//subscriptions/