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. Classi-
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.

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.

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. A recent post-mortem study further confirmed a potential pathogenic role of CRP in atheromatous plaque vulnerability, 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 chemoattraction 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. 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. Animal models, including transgenic mice, have also provided conflicting evidence regarding proatherogenic effects of CRP.

In contrast to the controversial results from in 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 years revealed a summary relative risk (RR) for CHD of 1.9 (95% confidence interval [CI], 1.5 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. A subsequent meta-analysis of 22 population–based studies, including a total of 7068 patients with incident coronary events, showed a similar result. 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, and lower CRP (upper tertile cut-off point of 2.0 mg/L, rather than 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, like smoking and hypertension did not have a significant incremental 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 analyzed; 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 studies and the Cardiovascular Health Study (CHS), an elderly population without a history of vascular disease at baseline. Thus, to date, 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 (RR/OR, 95% CI)*</th>
<th>Reference</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 (0.96–2.50)#</td>
<td>Am J Epidemiol. 1996;144:537–547</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 (1.8–4.8); Stroke: 1.9 (1.1–3.3)§</td>
<td>N Engl J Med. 1997; 336:973–979</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 (1.04–6.8)#</td>
<td>Arterioscler Thromb Vasc Biol. 1997; 17:1121–1127</td>
</tr>
<tr>
<td>Tracy et al</td>
<td>RRIP nested case-control</td>
<td>Elderly (65+)</td>
<td>CHD</td>
<td>3</td>
<td>145/146</td>
<td>M: 2.0 (0.6–6.4)**</td>
<td>Arterioscler Thromb Vasc Biol. 1997; 17:1121–1127</td>
</tr>
<tr>
<td>Ridker et al</td>
<td>WRS nested case-control</td>
<td>Healthy women</td>
<td>OVD</td>
<td>3</td>
<td>122/244</td>
<td>4.1 (1.7–9.8)#</td>
<td>Circulation. 1998; 96:731–733</td>
</tr>
<tr>
<td>Ageewall et al</td>
<td>Göteborg complete cohort</td>
<td>Hypertensive men</td>
<td>CHD</td>
<td>3</td>
<td>131</td>
<td>1.16 (0.86–1.28); (per 1 unit)</td>
<td>J Hypertens. 1998;16:537–541</td>
</tr>
<tr>
<td>Koenig et al</td>
<td>MONICA Augsburg complete cohort</td>
<td>Healthy men</td>
<td>CHD</td>
<td>8.2</td>
<td>936</td>
<td>1.50 (1.14–1.97); (per 1 SD)</td>
<td>Circulation. 1999; 99:327–324</td>
</tr>
<tr>
<td>Hanss et al</td>
<td>IOWA 65+ RHS case-cohort</td>
<td>Elderly (65+)</td>
<td>CV Mortality</td>
<td>4.6</td>
<td>178/499</td>
<td>1.8 (0.9–3.5)§</td>
<td>Am J Med. 1999;106:500–512</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 (0.81–4.60)#</td>
<td>Arterioscler Thromb Vasc Biol. 1999; 19:3071–3078</td>
</tr>
<tr>
<td>Gran et al</td>
<td>Grotthup nested case-control</td>
<td>Healthy M/F</td>
<td>CHD</td>
<td>12</td>
<td>133/258</td>
<td>RR not assessed; F=0.3346</td>
<td>J Intern Med. 2000;247:205–212</td>
</tr>
<tr>
<td>Mendall et al</td>
<td>Caephyll complete cohort</td>
<td>Healthy M/F</td>
<td>Stroke</td>
<td>5</td>
<td>1395</td>
<td>2.01 (1.4–3.6)#**</td>
<td>Eur Heart J. 2000;21:1584–1590</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 (0.67–3.13); F: 2.1 (1.19–3.8)#</td>
<td>Stroke. 2001; 32:2575–2579</td>
</tr>
<tr>
<td>Pero et al</td>
<td>Quebec CV complete cohort</td>
<td>Healthy men</td>
<td>CHD</td>
<td>5</td>
<td>2037</td>
<td>11.1 (0.7–16.6)</td>
<td>Arch Intern Med. 2001;161:2474–2480</td>
</tr>
<tr>
<td>Laine et al</td>
<td>Speedwell complete cohort</td>
<td>Healthy men</td>
<td>CHD</td>
<td>6.3</td>
<td>2055</td>
<td>1.60 (0.90–2.83)#</td>
<td>Arterioscler Thromb Vasc Biol. 2001;21:603–610</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>2.05 (0.79–6.83)#</td>
<td>Circulation. 2002; 105:2596–2599</td>
</tr>
<tr>
<td>Pradhan et al</td>
<td>WHI nested case-control</td>
<td>Healthy women</td>
<td>CHD</td>
<td>2.9</td>
<td>304/304</td>
<td>2.1 (1.1–4.1)#</td>
<td>J Am Med Assoc. 2002; 288:900–907</td>
</tr>
<tr>
<td>Luc et al</td>
<td>PRIME nested case-control</td>
<td>Healthy men</td>
<td>CHD</td>
<td>5</td>
<td>317/317</td>
<td>2.3 (1.1–4.0)†</td>
<td>Am J Med. 2002; 109:1575–1586</td>
</tr>
<tr>
<td>Van der Meer et al</td>
<td>Rotterdam nested case-control</td>
<td>Healthy men</td>
<td>CHD</td>
<td>5</td>
<td>157/500</td>
<td>1.2 (0.6–2.2)§</td>
<td>Arch Intern Med. 2003;163:1322–1328</td>
</tr>
<tr>
<td>Koenig et al</td>
<td>MONICA Augsburg complete cohort</td>
<td>Healthy men</td>
<td>CHD</td>
<td>6.6</td>
<td>345</td>
<td>2.21 (1.4–3.7)#</td>
<td>Circulation. 2004;109:1349–1353</td>
</tr>
<tr>
<td>Poi et al</td>
<td>NHS nested case-control</td>
<td>Healthy M/F</td>
<td>Stroke</td>
<td>8</td>
<td>239/469</td>
<td>1.53 (0.89–2.62)§</td>
<td>New Engl J Med. 2004;351:2599–2610</td>
</tr>
<tr>
<td>HRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cushan et al</td>
<td>OHS complete cohort</td>
<td>Healthy men</td>
<td>CHD</td>
<td>10</td>
<td>3871</td>
<td>1.37 (0.98–1.9)§</td>
<td>Circulation. 2005;112:25–31</td>
</tr>
<tr>
<td>Laksanas et al</td>
<td>KNH complete cohort</td>
<td>Healthy men</td>
<td>CV Mortality</td>
<td>14.6</td>
<td>1478</td>
<td>2.94 (1.46–5.91)#</td>
<td>Eur Heart J. 2005; 26:1783–1789</td>
</tr>
<tr>
<td>Wilson et al</td>
<td>PHS complete cohort</td>
<td>Healthy M/F</td>
<td>OVD</td>
<td>8</td>
<td>4446</td>
<td>1.16 (0.92–1.47)#</td>
<td>Arch Intern Med. 2005;165:2473–2478</td>
</tr>
<tr>
<td>Behskovdi et al</td>
<td>EPIC-Norfolk nested case-control</td>
<td>Healthy M/F</td>
<td>CHD</td>
<td>6</td>
<td>1108/2164</td>
<td>1.61 (1.31–2.12)#†</td>
<td>Arteriosclerosis. 2006;16:415–422</td>
</tr>
<tr>
<td>Koenig et al</td>
<td>MONICA/KORA case-control</td>
<td>Healthy M/F</td>
<td>CHD</td>
<td>11</td>
<td>382/180</td>
<td>M: 1.8 (0.28–2.77)‡</td>
<td>F: 1.35 (0.84–2.14)*</td>
</tr>
</tbody>
</table>

*Multivariable adjusted; †Partially adjusted; ‡Increase per 1 Unit or standard deviation (SD); #vs = < median; †Tertile analysis (T3 vs T1); #Quartile analysis (Q4 vs Q1); **Quintile analysis (Q5 vs Q1); $For CRP > 3 mg/L vs CRP < 1 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; RHP, Rural Health Promotion Project; WHS, Women’s Health Study; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; IOWA 65+ RHS, 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; NIH, 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.
stimulated by IL-6, express intercellular adhesion molecule-1 (ICAM-1) which participates in the proliferation of SMCs.15 In addition, ECs are able to stimulate macrophages to secrete MCP-1 and participate in the amplification of the inflammatory cascade15 through which IL-6 at least in part might exert its important function.

CRP. Still, its incremental predictive value above and beyond traditional risk factors, based on any of the available scores has not been definitely proven. Eventually, this issue might be solved on the basis of individual subject data in a meta-analysis currently prepared by the Emerging Risk Factors Collaboration Group. Until then widespread screening for CRP in unselected populations cannot be recommended.

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.15 IL-6 is able to stimulate macrophages to secrete MCP-1 and participates in the proliferation of SMCs.15 In addition, ECs stimulated by IL-6, express intercellular adhesion molecule-1 (ICAM-1).15 In a murine model of atherosclerosis, injection of excessive amounts of recombinant IL-6 resulted in enhanced fatty lesion development.16 Furthermore, IL-6 represents the principal procoagulant cytokine,17 but its most important function is the amplification of the inflammatory cascade15 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,18–19 in particular within the shoulder region of stable and unstable plaque, where it colocalized with the angiotensin II type 1 (AT-1) receptor.20 Uregulation 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.21 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.22

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.23 More importantly, the Fragmin and Fast Revascularization During Instability in Coronary Artery Disease II (FRISC II)24 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 population25–28 (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.29 Beyond induction of interferon (IFN)-γ20 with subsequent promotion of Th1 immune response, IL-18 enhances the expression of matrix metalloproteases (MMPs)31–33 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.31,34 In animal models, inhibition of IL-18 by IL-18 binding protein reduced atherosclerotic plaque development and progression in apoE-deficient mice35 and IL-18/apo-E double knockout mice exhibited reduced lesion size,36 thus further supporting a proatherogenic role of IL-18. In contrast, direct administration of IL-18 enhanced atherosclerosis in an IFN-γ-dependent manner,37 even in the absence of T-cells38 and induced/ promoted a switch to a vulnerable plaque phenotype by decreasing intimal collagen content and cap-to-core ratio.39

### Table 2. Interleukin-6 (IL-6) and Cardiovascular Disease: Overview of Prospective Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Design</th>
<th>Population</th>
<th>Outcome Variable</th>
<th>FU</th>
<th>No.</th>
<th>Risk Estimate (β/95% CI)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harris et al</td>
<td>Iowa 65+RHS</td>
<td>Elderly 65+</td>
<td>CV Mortality</td>
<td>4.6</td>
<td>176/499</td>
<td>2.2 (1.0–4.6)</td>
<td>Am J Med. 1999;106:506–512</td>
</tr>
<tr>
<td>Ridker et al</td>
<td>PHS</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>
<tr>
<td>Volpato et al</td>
<td>WHAS</td>
<td>Elderly 65+</td>
<td>CV Mortality</td>
<td>3</td>
<td>629</td>
<td>2.52 (2.1–4.5)</td>
<td>Circulation. 2001;103:947–953</td>
</tr>
<tr>
<td>Cesari et al</td>
<td>Health ABC</td>
<td>Healthy women</td>
<td>CHO/Stroke</td>
<td>3.6</td>
<td>2225</td>
<td>2.1 (1.1–4.0)</td>
<td>J Am Med Assoc. 2002; 288:960–987</td>
</tr>
<tr>
<td>Lowe et al</td>
<td>WOSCOPS</td>
<td>Dyslipidemic men</td>
<td>1.64 (1.1–2.40)</td>
<td>Arterioscler Thromb Vasc Biol. 2004; 24:1529–1534</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

To date, 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 up-regulates 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 pro-inflammatory 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 its 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 or 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 prostanoïd 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 A2

Lipoprotein-associated phospholipase (Lp-PLA2) represents another emerging biomarker for atherosclerotic disease and is presently under intensive investigation. Lp-PLA2, a 45.4-kDa protein, is a calcium-independent member of the phospholipase A2 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-PLA2 plasma isofrom circulates primarily bound to low-density lipoproteins (LDL), the other third is distributed between HDL and very low-density lipoproteins (VLDL). Lp-PLA2 may promote oxidation of LDL, and recent investigations have stressed the proatherogenic properties of this enzyme. LDL provides a circulating reservoir, in which Lp-PLA2 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 and proatherogenic mediators, namely lysophosphatidylcholine (LysoPC) and oxidized fatty acid (oxFA).⁶⁶ LysoPC is a potent chemoattractant for T-cells and monocytes, promotes endothelial cell dysfunction, stimulates macrophage proliferation, and induces apoptosis in SMCs and macrophages.⁶⁴ Thus, Lp-PLA₂ may represent an important “missing link” between the oxidative modification of LDL in the intimal layer of the arterial wall and local inflammatory processes within the atherosclerotic plaque.

Several studies in initially healthy subjects but also in those with manifest atherosclerosis have found an association between increased concentrations of Lp-PLA₂ and future coronary and cerebrovascular events, independent of a variety of potential confounders⁶⁷–⁷⁶ (Table 3). However, measurement of Lp-PLA₂ in the early phase of the ACS was not associated with increased risk for recurrent events.⁷¹–⁷³ Apart from an important role of Lp-PLA₂ in the prediction of future CV disease, this enzyme could also represent a therapeutic target, because initial studies have demonstrated a significant clinical benefit by inhibiting this enzyme through azetidinones, a new class of compounds acting as novel therapeutic agents.⁷⁴–⁷⁶ Furthermore, circulating sPLA₂-II in blood has been demonstrated to predict cardiovascular events in initially healthy subjects and in patients with manifest ACS either in the prediction of future CV disease, this enzyme could also represent a therapeutic target, because initial studies have demonstrated a significant clinical benefit by inhibiting this enzyme through azetidinones, a new class of compounds acting as novel therapeutic agents.⁷⁴–⁷⁶

### Table 3. Lp-PLA₂ and Cardiovascular Disease: Overview of Prospective Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Design</th>
<th>Population</th>
<th>Outcome Variable</th>
<th>FU</th>
<th>No.</th>
<th>Risk Estimate (RR/OD, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packard et al†</td>
<td>WOSCOPS nested case-control</td>
<td>Dyslipidemic men</td>
<td>CHD</td>
<td>4.9</td>
<td>500/1160</td>
<td>1.18 (1.05–1.33)</td>
</tr>
<tr>
<td>Blake et al†</td>
<td>WHS nested case-control</td>
<td>Healthy women</td>
<td>CHD</td>
<td>3</td>
<td>123/123</td>
<td>1.17 (0.85–3.35)</td>
</tr>
<tr>
<td>Ballantyne et al</td>
<td>ARIC nested case-cohort</td>
<td>Healthy MF</td>
<td>CHD</td>
<td>6–8</td>
<td>606/740</td>
<td>1.15 (0.81–1.63)</td>
</tr>
<tr>
<td>Koenig et al†</td>
<td>MONICA complete cohort</td>
<td>Healthy men</td>
<td>CHD</td>
<td>14</td>
<td>934</td>
<td>1.21 (1.01–1.45)</td>
</tr>
<tr>
<td>Oel et al‡</td>
<td>Rotterdam nested case-control</td>
<td>Elderly (55+)</td>
<td>CHD</td>
<td>10</td>
<td>377/1822</td>
<td>1.76 (1.09–2.85)</td>
</tr>
<tr>
<td>Persson et al‡</td>
<td>Malmö complete cohort</td>
<td>Healthy MF</td>
<td>CVD</td>
<td>9.4</td>
<td>145/4938</td>
<td>1.69 (1.10–2.60)</td>
</tr>
<tr>
<td>Britakis et al‡</td>
<td>Mayo complete cohort</td>
<td>CHD patients</td>
<td>CVD</td>
<td>4</td>
<td>504</td>
<td>1.30 (1.06–1.59)</td>
</tr>
<tr>
<td>Horne et al‡</td>
<td>IHCS complete cohort</td>
<td>CHD patients</td>
<td>CHD death</td>
<td>6.7</td>
<td>1493</td>
<td>1.73 (0.84–3.81)</td>
</tr>
<tr>
<td>Oel et al‡</td>
<td>Rotterdam nested case-control</td>
<td>Elderly (55+)</td>
<td>Stroke</td>
<td>12</td>
<td>200/1822</td>
<td>1.77 (1.19–2.64)</td>
</tr>
<tr>
<td>Ballantyne et al</td>
<td>ARIC nested case-cohort</td>
<td>Healthy MF</td>
<td>Stroke</td>
<td>4.4</td>
<td>194/766</td>
<td>1.93 (1.14–3.27)</td>
</tr>
<tr>
<td>Persson et al‡</td>
<td>Malmö complete cohort</td>
<td>Healthy MF</td>
<td>Stroke</td>
<td>9.4</td>
<td>112/4938</td>
<td>1.69 (0.90–3.18)</td>
</tr>
<tr>
<td>Koenig et al‡</td>
<td>KAROLA complete cohort</td>
<td>CHD patients</td>
<td>CVD</td>
<td>4</td>
<td>1,051</td>
<td>2.09 (1.10–3.96)</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); ‡ Quartile 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, Langzeittherapie der kardiologischen Anschlussbehandlung; IHCS, Intermountain Heart Collaborative Study; NOMAS, Northern Manhattan Study.

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

### 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.⁷⁷–⁷⁹

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.85 There are several pathways through which MPO could exert its deleterious effects. MPO together with other enzymes such as lipoxigenase 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.85 MPO could be also involved in the development of endothelial dysfunction, because MPO uses the atheroprotective endothelial-derived NO as a substrate.86 Nonetheless, a most pivotal characteristic of MPO and its end product HOCl remains its ability to activate MMPs87 and deactivate inhibitors of MMPs,88 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).89 In a large cohort of patients with chest pain, a single measurement of MPO on admission independently predicted acute MI.90 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).91 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.91 MMPs are widely expressed in monocytes/macrophages, ECs and SMCs, fibroblasts, and neoplastic cells.92 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.91 Recently, several lines of evidences have demonstrated that MMPs play an important role in atherosclerosis.92 Most importantly, MMPs are highly expressed in macrophage-rich areas of the atherosclerotic plaque, especially at the shoulder region of the cap,93 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.94–96 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.97 Interestingly, high concentrations of the endogenous tissue inhibitors of metalloproteinase-1 (TIMP) were also predictive for future CV death in this study,98 which has been confirmed by others.99 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 CD40L100 and thus MCP-1 expression is increased in atherosclerotic lesions,101 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.102 Activation of the MCP-1/CCR2 pathway has also been shown to induce expression of MMPs,103 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,104 and anti-monocyte MCP-1 gene therapy limited the progression and destabilization of established atherosclerosis in apolipoprotein E-knockout mice.105 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 Orbofibin 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.106 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.107 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).108 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 tissue108 and was found to be upregulated within early and advanced atherosclerotic lesions.109 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 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-
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 suggested136,137 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 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


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

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/