Atherosclerosis is characterized by a non-specific local inflammatory process accompanied by a systemic response. A number of prospective studies in initially healthy subjects and in patients with manifest atherosclerosis have now convincingly demonstrated a strong and independent association between even slightly elevated concentrations of various systemic markers of inflammation and a number of cardiovascular endpoints (see Table 1). Measurements of inflammatory markers might also add to the predictive value of atherogenic lipoprotein phenotyping in assessing long-term coronary risk. This suggests that the evaluation of the ‘active’ inflammatory state of patients with manifest atherosclerosis yields important complementary prognostic information.

Currently, C-reactive protein (CRP), the classical acute-phase protein, seems to be the marker of choice for the clinical situation. However, there are other emerging biomarkers, such as lipoprotein-associated phospholipase A2 (Lp-PLA2), oxidized low-density lipoprotein (oxLDL), interleukin (IL)-18 and adiponectin, that might improve our ability to identify patients at risk for future coronary heart disease (CHD).

**CRP**

Several studies published during the past decade have provided strong evidence that CRP, although a classical acute-phase reactant with a relatively short half-life (about 19 hours), represents a reliable long-term marker of cardiovascular risk. So far, the results from more than 25 different prospective studies have been reported, and they clearly demonstrate a significant and independent association between increased concentrations of CRP and future cardiovascular events. Furthermore, the recent American Health Association (AHA)/US Centers for Disease Control and Prevention (CDC) consensus report recommend the measurement of CRP in asymptomatic subjects at intermediate risk for future coronary events (10-year risk of 10% to 20%) and in selected patients after an acute coronary syndrome. This recommendation was based on the fact that the current inflammatory markers identified, high-sensitivity (hs)-CRP has the analyte and assay characteristics most conducive to use in practice (class 2a, level of evidence B), and that other inflammatory markers should not be measured for determination of cardiovascular (CV) risk in addition to hs-CRP (class 3, level of evidence C).

Since an important issue in risk assessment relates to the potentially clinically relevant incremental information conveyed by a new risk-marker, several studies have revealed that the addition of CRP to the conventional lipid profile improved risk prediction from a clinical standpoint. The authors have been able to demonstrate that the potential of CRP measurement might modify risk prediction based on the Framingham Risk Score (FRS), a score that is recommended for global risk assessment in subjects prone to CHD. The authors compared the proportions of incident coronary events within 10 years estimated by the Cox model for the five categories of the FRS alone (see Figure 1, left panel) and for different CRP categories in each category of FRS, adjusted for survey and components of the FRS (see Figure 1, right panel). Probability values of the stratified analyses are also given in Figure 1 (right panel, above each FRS category). Cox regression revealed a considerable modification in coronary event incidence based on CRP concentrations and, more importantly, in particular, in categories of FRS associated with a 10% to 20% risk per 10 years, elevated concentrations of CRP were consistently and statistically significantly associated with a further increased risk (p=0.03 and p=0.02, respectively). In contrast, in men with a risk <6% or 6% to 10% and >20% over 10 years, CRP had no statistically significant additional effect on the prediction of a first coronary event. In receiver operating characteristic (ROC) analyses, regarding the different areas under the curve (AUC), a remarkable increase was found for the intermediate FRS categories of 11% to 14% and 15% to 19% (increase in the AUC from 0.725 to 0.776 and from 0.695 to 0.751, respectively). Thus, there is initial evidence that CRP measurement may aid in improving risk prediction in a population for which there is otherwise considerable uncertainty about future prognosis. However, such data have to be confirmed in other populations before recommendations for widespread use of this marker can be made.
Lp-PLA₂

More recently, attention has focused on Lp-PLA₂, an enzyme that may directly promote atherosclerosis by generating potent pro-inflammatory and pro-atherogenic products, like lysophosphatidylcholine (lysoPC) and oxidized free fatty acids (oxFFA). Lp-PLA₂ is produced mainly by cells critically involved in atherosclerotic plaque development, such as monocytes, macrophages, T-lymphocytes, and mast cells. Lp-PLA₂ has been found to be upregulated in atherosclerotic lesions, especially in complex plaques, as well as in the fibrous cap of coronary lesions prone to rupture. Two-thirds of Lp-PLA₂ circulates primarily bound to LDL, the other third is distributed between high-density lipoprotein (HDL) and very-low-density lipoprotein (VLDL). Lp-PLA₂ seems to play a dual role in atherosclerotic disease. While the HDL-associated enzyme is considered to be protective against atherosclerosis, Lp-PLA₂ bound to LDL possesses potential pro-atherogenic properties, which are mainly associated with its ability to hydrolyze oxLDL with subsequent release of potent pro-inflammatory mediators such as LysoPC and oxFFA. Upon release from oxLDL, as a result of cleavage of a short acyl group of oxidized phosphatidylcholine at the sn-2 position, these compounds trigger a cascade of events that might directly promote atherogenesis. 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.

Hitherto published results from several prospective studies conducted in initially healthy subjects from various populations revealed an independent association between increased concentrations of Lp-PLA₂ and future cardiovascular events (see Table 2). Initial evidence for such an association came from the West of Scotland Coronary Prevention Study (WOSCOPS), in which the relative risk (RR) associated with one standard deviation (SD) increase in Lp-PLA₂ was 1.18 (95% confidence interval (CI), 1.05–1.33; p=0.005) after controlling for traditional risk factors, and was independent of various other biomarkers, such as CRP, leukocyte count and fibrinogen. Almost identical results have been found in the MONitoring trends and determinants in CArdiovascular diseases (MONICA) studies, with approximately 20% elevated coronary risk per one SD increase in Lp-PLA₂. The predictive role of Lp-PLA₂ has also been assessed in the Women’s Health Study (WHS), a large cohort of middle-age normcholesterolemic women. However, elevated levels of Lp-PLA₂ did not predict subsequent CV events in this low-risk population for CV disease (CVD). This lack of association could be attributed to existing gender differences for Lp-PLA₂ and a problem with the power in this study. Results from the Atherosclerosis Risk in Communities (ARIC) study demonstrated only a borderline association in the total case-cohort; however, in subjects with low LDL cholesterol, Lp-PLA₂ significantly and independently predicted CHD, thereby suggesting that it might be a useful marker for identifying high-risk patients with relatively normal levels of LDL cholesterol, a group in whom additional markers of risk are clearly needed. Finally, the potential additive value of Lp-PLA₂ to CRP in predicting CHD risk has been tested in several studies. Taken together, these data suggest that Lp-PLA₂ and CRP may be complementary (or additive) in identifying high-risk subjects and, therefore, the combination of both markers may further improve risk assessment. What is still missing is information on the potential additive prognostic information of Lp-PLA₂ in the context of global risk assessment using the FRS.

### Table 1: Inflammatory Biomarkers Investigated Prospectively in Epidemiological Studies

<table>
<thead>
<tr>
<th>Non-protein markers</th>
<th>Frequently studied proteins</th>
<th>Infrequently studied proteins</th>
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<tbody>
<tr>
<td>Leukocytes</td>
<td>C-reactive protein</td>
<td>Oromucoid</td>
</tr>
<tr>
<td>ESR</td>
<td>Serum amyloid A</td>
<td>Alpha-antitrypsin</td>
</tr>
<tr>
<td>Plasma viscosity</td>
<td>Fibrinogen</td>
<td>Haptoglobin</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>Ceruloplasmin</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>C3, C4</td>
<td></td>
</tr>
<tr>
<td>PAI-1</td>
<td>IgA, G, M, and E</td>
<td></td>
</tr>
<tr>
<td>vWF</td>
<td>Sialic acid</td>
<td></td>
</tr>
<tr>
<td>D-Dimer</td>
<td>CIC</td>
<td></td>
</tr>
<tr>
<td>Cytokines (IL-6, 8, 18)</td>
<td>Lp (a), oxLDL</td>
<td></td>
</tr>
<tr>
<td>CAMs</td>
<td>Lp-PLA₂, sPLA₂-IIA</td>
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### Figure 1: Occurrence of a First Coronary Event Within 10 Years, Estimated by Cox Proportional Hazards Models in Percentages

Framingham Estimate of 10-year Risk

Left: percentage estimated by a model with FRS (five categories) adjusted for survey. Right: percentage estimated for each of five FRS categories by a model with CRP (three categories) adjusted for FRS (continuous) and survey. Probability values indicate significance status of CRP in the Cox model.
Oxidized LDL

The oxidative modification hypothesis of atherogenesis proposes that the most significant event in early lesion formation is lipid oxidation, placing oxLDL in a central role for the development of this disease. OxLDL has a large number of biological actions and consequences, including injuring endothelial cells, expressing adhesion molecules, recruiting leukocytes and retaining them, and the formation of foam cells. Pro-atherogenic properties of oxLDL are summarized in Table 3. 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 expression in human vascular endothelial cells and in monocyte-derived macrophages. It has been also shown that oxLDL upregulates the expression of MMP-1 and -3 in human coronary artery endothelial cells, an effect mediated through its endothelial receptor, lectin-like oxidized LDL receptor (LOX)-1. Furthermore, oxLDL triggers the CD40/CD40 ligand (CD40L) signaling pathway, which also might lead to a pro-inflammatory reaction and endothelial injury.

More recently, the authors have reported results from a nested case–control study that was conducted within two population-based MONICA/KORA Augsburg surveys. The association between plasma oxLDL and risk of future CHD was investigated in 88 middle-aged men with an incident CHD event and 258 age-matched controls during a mean follow-up of 5.6 years. Baseline oxLDL concentrations were significantly higher in CHD cases compared with controls. After multivariable adjustment, plasma oxLDL was the strongest predictor of CHD events compared with a conventional lipoprotein profile and other traditional risk factors for CHD, with the hazard ratio (HR) for a future CHD event being 4.25 (95% CI, 2.09–8.63; p<0.001) if the top tertile of the oxLDL distribution was compared to the bottom tertile. Furthermore, the authors assessed whether the predictive value of oxLDL was additive to other risk factors of CHD and found that oxLDL significantly improved prediction of incident CHD in addition to the other cardiovascular risk factors. Finally, when both oxLDL and CRP were simultaneously assessed in the same model, they still predicted future coronary events, even after multivariable adjustment including the total cholesterol (TC)/HDL cholesterol (HDL-C) ratio, one of the most powerful predictors of risk among conventional lipid variables. However, this study was the first prospective study conducted in apparently healthy men from an area with moderate absolute risk of CHD. Thus, further studies are warranted to establish the clinical relevance of oxLDL measurement in various stages of the atherosclerotic process and to identify the specific pathophysiological mechanisms by which oxLDL exerts its deleterious effects.

IL-18

IL-18, a new member of the IL-1 family of cytokines, has recently been suggested to play an important role in the regulation of innate and adaptive immunity. Beyond induction of interferon (IFN) (with subsequent promotion of T-helper cell type 1 (Th1) immune response), IL-18 also enhances the expression of MMPs and both these capacities of IL-18 characterize it as a crucial and potent mediator of atherosclerotic plaque destabilization and vulnerability. Some experimental studies have demonstrated increased expression of IL-18 in human atherosclerotic plaque, especially in lesions prone to rupture. Animal models further support the pro-atherogenic role of IL-18, demonstrating that endogenous inhibition of IL-18 by IL-18 binding protein reduced plaque development and progression in apoE-deficient mice. Direct administration of IL-18, in contrast, enhances atherogenesis and promotes a switch to a vulnerable plaque phenotype by decreasing intimal collagen content and cap-to-core ratio.

While experimental studies on the role of IL-18 in atherogenesis seem to be consistent and very promising, the epidemiological evidence remains scarce and somewhat controversial. To date, only one study has assessed the prognostic value of elevated IL-18 for future coronary events (follow-up period, five years) in apparently healthy subjects within the PRIME cohort, comprising 10,600 middle-aged men without pre-
Table 2: Pro-atherogenic Properties of Oxidized LDL

<table>
<thead>
<tr>
<th>Biological effect</th>
<th>Possible mechanism</th>
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<tbody>
<tr>
<td>Foam cell formation</td>
<td>Direct uptake of cholesterol by scavenger receptors as well as inhibition of their export from macrophages</td>
</tr>
<tr>
<td>Chemoattraction of monocytes, T-lymphocytes</td>
<td>Increased expression of MCP-1 and direct chemotactic effect</td>
</tr>
<tr>
<td>Macrophage trapping within the intima</td>
<td>Inhibition of the motility of macrophages</td>
</tr>
<tr>
<td>Impaired vascular function (vasoconstrictor effect)</td>
<td>Inhibition of nitric oxide release or function</td>
</tr>
<tr>
<td>Adhesion of monocytes to endothelium</td>
<td>Increased expression of adhesion molecules</td>
</tr>
<tr>
<td>Plaque rupture</td>
<td>Enhanced formation of matrix metalloproteinases</td>
</tr>
<tr>
<td>Cell proliferation</td>
<td>Induction of growth factors</td>
</tr>
<tr>
<td>Thrombogenesis</td>
<td>Promotion of platelet aggregation and increased tissue factor activity</td>
</tr>
<tr>
<td>Increased cellular death</td>
<td>Induction of Fas-mediated apoptosis</td>
</tr>
<tr>
<td>Induction of pro-inflammatory genes</td>
<td>Activation of nuclear factor-B</td>
</tr>
<tr>
<td>Increased antigenicity</td>
<td>Induction of autoantibody (IgG) formation</td>
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Adiponectin

Among numerous emerging pro-inflammatory and pro-atherogenic biomarkers, adiponectin seems to be a novel marker of anti-atherogenicity. Adiponectin, a 244 amino acid collagen-like protein and a member of a new family of obesity-related hormones, the adipocytokines, which is produced solely by white adipose tissue, may be linked to both insulin resistance and endothelial dysfunction.34 Apart from its role as an insulin sensitizing agent, and its implication in metabolic disorders, adiponectin might also be involved in the regulation of inflammatory processes that contribute to atherosclerosis by, for instance, the inhibition of expression of adhesion molecules and by preventing the attachment of monocytes to the endothelial surface.35 Low adiponectin levels are associated with reduced expression of nitric oxide and increased expression of angiotensin II from the endothelium.36 All these properties of adiponectin are in support of a promising role for this molecule as an anti-inflammatory and anti-atherogenic biomarker for the prediction of future CV events.

Only one prospective study so far, conducted by Pischon and colleagues36 in US health professionals, has assessed the predictive value of adiponectin for future coronary events in apparently healthy, non-diabetic subjects. The authors found a significantly reduced risk of subsequent acute myocardial infarction (MI) associated with higher levels of adiponectin in serum at baseline; notably, this association was also reduced after adjustment for covariates, but persisted after inclusion of lipid variables in the model, including HDL cholesterol.

The authors also studied the relative risk for future CHD events associated with reduced levels of adiponectin during a 14-year follow-up.37 Higher adiponectin levels were associated with considerably lower risk of about 40% to 50% for a first-ever acute CHD event and this association was independent of a variety of potential confounders. In a Cox model, the HR of a future coronary event, comparing the top tertile of the adiponectin distribution with the bottom tertile, was 0.54 (95% CI, 0.31–0.93, after adjustment for CV risk factors). Only the introduction of HDL-C in the model resulted in a moderate attenuation of the association, which then became borderline significant. Thus, these data suggest an antiatherogenic role of increased concentrations of adiponectin and that hypo-adiponectinemia might therefore be associated with an increased risk of atherosclerotic disease. The protective effect of high serum concentrations might, at least in part, be explained through its strong positive correlation with HDL-C.

existing CHD from France and Belfast.31 Elevated IL-18 concentrations at baseline were associated with a two-fold increased risk for subsequent coronary events after multivariable adjustment in a nested case-control design. However, the association was found only when data from both populations were pooled for analysis. When the prognostic value of IL-18 was investigated in the French and Irish cohorts separately, the significance of the association was lost in France, whereas it became even more pronounced in the Irish population.

The authors have attempted to replicate these findings and conducted a large case–cohort study in initially healthy middle-aged men and women based on data from the MONICA/KORA Augsburg studies collected between 1984 and 2002 (mean follow-up, 11 years).32 Concentrations of IL-18 were measured in 382 case subjects (295 men and 87 women) with incident CHD and in 1980 non-case subjects (1,009 men and 971 women). In crude and in age- and survey-adjusted analyses, there was a statistically significant association between increased concentration of IL-18 and incident CHD in men, whereas no significant association was seen in women. However, after multivariable adjustment for cardiovascular risk factors and the total cholesterol/HDL ratio, C-Reactive Protein and IL-6, this association was attenuated, no longer statistically significant in men and remained non-significant in women (HR, 95% CI, 1.20 (0.85–1.69) and 1.25 (0.67–2.34), respectively). Thus, elevated concentrations of IL-18 were not statistically significantly associated with risk of CHD in men and women from an area with moderate absolute risk of CHD. This large population-based case–cohort study therefore suggests that IL-18 may only serve as a marker of future cardiovascular events in men with manifest CHD and/or in areas of high absolute risk of CHD.
Summary and Conclusion

During the past decade, an increasing number of novel biomarkers of cardiovascular risk have been identified. To be implemented into clinical practice, they should fulfill certain requirements, such as providing independent information on risk prediction in addition to global risk assessment, be reliable and easily reproducible, and show high sensitivity and specificity. Finally, simple and robust assays should be commercially available.

In the future, we will probably see a biomarker profile that covers various aspects of the complex pathophysiology of the atherothrombosis process; rather than single markers only, and, finally, testing the 'inflammation hypothesis' in large clinical trials will represent an important goal for clinical research on atherosclerosis.

References